Science Library

MAY 5 1948

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XXXIV HÄFT. 3

LUND 1948, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in heredity, is published by the MENDELIAN SOCIETY at Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. Each volume contains about 450 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Twenty-five (25) Swedish Kronor per volume.* Post free. (The price of Vols. I—X is 10 Swedish Kronor per volume, of Vols. XI—XXIII, 15 Sw. Kr., and of Vols. XXIV—XXV 20 Sw. Kr.)

ROBERT LARSSON
Editor of »Hereditas»
3 Adelgatan, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *ARNE MÜNTZING*
PROFESSOR, FIL. DR. *HERIBERT NILSSON*
PROFESSOR, FIL. DR. *GERT BONNIER*
PROFESSOR, MED. DR. *ERIK ESSEN-MÖLLER*

THE RELATION BETWEEN CHROMOSOME CONSTITUTION AND SEX IN THE OFFSPRING OF TRIPLOID MELANDRIUM

BY *M. WESTERGAARD*

LABORATORY OF GENETICS, ROYAL VETERINARY AND AGRICULTURAL COLLEGE, COPENHAGEN, DENMARK

I. INTRODUCTION.

EXPERIMENTALLY produced polyploid strains of the dioecious plant *Melandrium album* have been a valuable tool for the study of sex genetics in this species (for references, see WARMKE, 1946; WESTERGAARD, 1946). The experiments have shown that the *Y* chromosome is male determining. Three different sections of *Y* have been mapped by means of spontaneous fragmentations, each section playing a distinct part in sex determination by suppressing femaleness and promoting maleness (WESTERGAARD, 1946; cf. also Fig. 2 in the present paper). The *X* chromosome of *Melandrium* is female determining (cf. WARMKE, 1946). These conclusions are mostly based on the study of sex expression in euploid *Melandrium* strains (2 *A*—3 *A*—4 *A* plants) with various combinations of sex chromosomes. From crossings between triploids it is possible to obtain plants with aneuploid autosome numbers and different combinations of sex chromosomes. The sex expression in such plants might give interesting information with respect to the significance of the autosomes in sex determination. The results of triploid *Melandrium* crossings have earlier been published by WARMKE and BLAKESLEE (1940). Since these authors obtained male and female plants only in the aneuploid material, they concluded (cf. WARMKE, 1946) that the autosomes are of no importance in the mechanism of sex determination of *Melandrium*.

The present paper deals with the Danish progeny of triploid *Melandrium* crossings. The results described are strikingly different from those obtained by the American authors, and the material does not support the statement that the autosomes are insignificant for the sex determination of this species.

The investigations were carried out at the Laboratory of Genetics of the Agricultural College, Copenhagen.

II. EXPERIMENTAL PART.

1. *Crossings between triploids.* — The theoretical ratios to be expected from the cross 3n *XXX* ♀ × 3n *XXY* ♂ have been reported previously (WESTERGAARD, 1940, p. 65). Owing to the fact that *XXY* males form a great excess of *X* and *XY* gametes and only a small number of *XX* and *Y* gametes, the majority of the offspring plants will have the sex chromosome constitutions *XX*—*XXY*—*XXX* or *XXXY*, but a small proportion of *XY* and *XXXX* plants may also appear. The autosome numbers may vary between 22 (the diploid number) and 44 (the tetraploid number) chromosomes, but, if triploid gametes should be formed occasionally, plants with up to 66 autosomes may be present.

The results of the crossings are given in Table 1. On account of a very poor seed germination, only 46 plants were secured. The chromosome numbers of this and the following samples were counted in root tip mitoses. The sex chromosome constitution of the females was likewise worked out from root tips, whereas the sex chromosomes of the males and hermaphrodites were counted on P.M.C. smears. The cytological technique has been described in previous publications.

The aneuploid plants group-

TABLE 1. *3A* + *XXX* ♀ × *3A* + *XXY* ♂.

Sex	Sex chromosomes	Autosome numbers 24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Total
♀♀	*XX*						1				2				1			1			1				6
	XXX	2			1		1				1	1		1	1		2	1	1			1			13	21
	XXXX																			2					2	
♂♂	*XXY*												1			1									1	10
	XXXY									1				1		3	1	1		1	1[1]		1		9	
♂♂	*XXY*									1		1		1			1	1							1	15
	XXXY								1					3	1		2	2		1					10	
Total		2	—	—	1	—	2	—	—	1	4	2	1	5	3	4	6	6	1	4	1	2	—	1	46

[1] The *Y* chromosome was fragmented.

ed in Table 1 show a very conspicuous morphological variation; since, however, the investigation of the sex expression is our main object, only this variation has been recorded. Table 1 comprises 10 intersexual

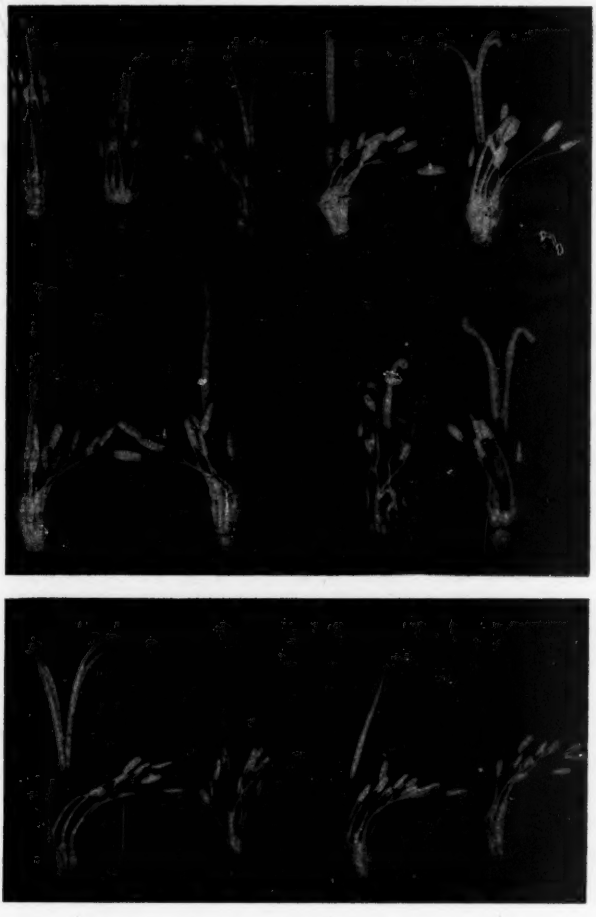


Fig. 1. — *a*. 9 intersexual offspring plants from crosses between triploids (cf. Table 1). — *b*. 4 flowers from the same intersexual plant, showing different degrees of intersexuality. — × 1 1/2.

plants in addition to 21 females and 15 males. One of the intersexual plants proved to have a fragmented *Y* chromosome [the Y^1 plant S. 443—9 described previously (WESTERGAARD, 1946)]. The nine other

plants had a normal *Y* chromosome in addition to three (or, in one case, only two) *X* chromosomes and varying numbers of autosomes.

The plant with the fragmented *Y* chromosome was a typical »euhermaphrodite», viz. a normal bisexual plant with both anthers and gynoecia normally developed in all flowers. The 9 other intersexes, however, were »androhermaphrodites», viz. much more male-like intersexes with only slightly developed female organs, especially in the later flowers. At the end of the growing season most flowers were pure males (Fig. 1, *a* and *b*). Despite the abnormal development of the female organs seeds could be secured by selfing all 9 plants.

The autosome numbers of the triploid offspring plants cover almost the whole range of variation between 2n and 4n and most plants have chromosome numbers above the triploid number (33 chromosomes). The same tendency is found in the material published by WARMKE and BLAKESLEE (1940). Obviously, the triploid *Melandrium* crossings behave differently from most other triploid crossings where, most frequently, only trisomic or secondary trisomic gametes are functioning. It should also be noted that the intersexual plants are found among the plants with the highest autosome numbers.

2. *Crossings between triploids and diploids.* — From the cross 2n *XX* ♀ × 3n *XXY* ♂ most of the offspring will have the sex chromosomes *XX* and *XXY*, though a few *XXX* and *XY* plants are also expected. The reciprocal cross 3n *XXX* ♀ × 2n *XY* ♂ results in *XX*—*XXX*—*XY* and *XXY* plants in equal proportions (WESTERGAARD, 1940, p. 65). Autosome numbers between 22 and 33 are expected, but some tetraploids (with 44 autosomes) may arise from triploid gametes. The results of the crossings are recorded in Tables 2 and 3; the autosome numbers in the functioning gametes from triploid parents are given in the bottom rows. The crossings produced only male and female plants; no intersexes appeared.

TABLE 2. *2n XX* ♀ × *3n XXY* ♂.

Sex	Sex chromosomes	Autosomes										Total
		22	23	24	25	26	27	28	29	30	31	
♀♀	*XX*	3	9	6	1	3	1	2			1	26
♂♂	*XY*									1		1
	XXY		1						1	1	1	4
	Total	3	10	6	1	3	1	2	1	2	2	31
Gametes from 3n		x	x+1	x+2	x+3	x+4	x+5	x+6	x+7	x+8	x+9	

TABLE 3. *3n XXX* ♀ × *2n XY* ♂.

Sex	Sex chromosomes	Autosomes 23	24	25	26	27	28	29	30	Total
♀♀	*XX*	2	1		3	1	1		1	9
	XXX	1		1	1					3
♂♂	*XY*	3	1	2	1		2	3	2	14
	XXY		2	1				1		4
	Total	6	4	4	5	1	3	4	3	30
Gametes from 3n		x+1	x+2	x+3	x+4	x+5	x+6	x+7	x+8	

3. *Crossings between triploids and tetraploids.* — A tetraploid *XXXX* ♀ × triploid *XXY* ♂ is expected to give rise to the sex chromosome combinations *XXX* and *XXXY* in excess, together with a small number of *XXXX* and *XXY* types. The autosome numbers will vary from 33 to 44 chromosomes. 16 plants were produced from the cross-

TABLE 4. *4n XXXX* ♀ × *3n XXY* ♂.

Sex	Sex chromosomes	Autosomes 34	35	36	37	38	39	40	41	42	43	44	Total
♀♀	*XXX*		2	1	1								4
♂♂	*XXXY*								1	1	2		4
♂♂	*XXXY*	1		1	1	3		1				1	8
	Total	1	2	2	2	3		1	1	1	2	1	16
Gametes from 3n		x+1	x+2	x+3	x+4	x+5	x+6	x+7	x+8	x+9	x+10	2x	

ing (Table 4) and, this time, four intersexual plants appeared, which were androhermaphrodites with a normal *Y* chromosome, similar to the 9 plants depicted in Fig. 1. Also in this case, the intersexes appear among the plants with most autosomes.

4. *First generation offspring of the triploid intersexes.* — The progeny of only two out of the 9 intersexes were followed through several generations. The first plant, S. 440—6, had the chromosome number 38 + *XXXY*. By selfing it gave rise to 48 plants (S. 475). The relation between chromosome constitution and sex in this sowing is shown in Table 5. Three sex chromosome types, viz. 1 *XXXX* : 2 *XXXY* : 1 *XXYY*, are to be expected when an *XXXY* plant is selfed; however, the observed ratio (16 : 21 : 5) deviates significantly from this expectation ($\chi^2 = 11{,}0$, $P < 0{,}01$). This deviation may possibly be ex-

plained by certation between the different pollen types in the stigma. The six plants with 3 or 5 sex chromosomes must arise from fertilization with aneuploid gametes which are likely to be formed from non-disjunction of the sex chromosomes.

As to sex expression, the table shows that the intersexual plant gives rise to both females, hermaphrodites and males, the two latter groups having one or two *Y* chromosomes. The intersexes are again of the androhermaphroditic type. However, at least 6 out of the 21 intersexual plants were classified as more intermediary bisexual plants with better developed female organs. In these six plants the majority of the flowers were intersexual even at the end of the growing season. The offspring of some of the intersexual plants will be described in the following section.

TABLE 5. *Sowing number 475, S. 440—6, selfed.*

Sex	Sex chromosomes	Autosomes												Total	
		36	37	38	39	40	41	42	43	44	45	46	47		
♀♀	*XXXX*		1		3		3	6	1	2				16	17
	XXX							1						1	
♀♂	*XXXY*	1		1	2	3	2	3	4		1			17	21
	XXXXY									1				1	
	XXYY								1	1				2	
	XXXYY			1										1	
♂♂	*XXY*						1	1					1	3	10
	XXXY					1		2	1					4	
	XXYY			1		1						1		3	
	Total	1	1	3	5	5	6	13	7	4	1	1	1		48

The second intersexual plant (S. 441—1) had the chromosome number $2n = 42 + XXXY$. By selfing it gave rise to 7 *XXXX* ♀♀ : 1 *XXX* ♀ : 8 *XXXY* ♀♂ : 1 *XXYY* ♂ : 10 *XXXY* ♂♂ : 4 *XXYY* ♂♂ : 1 *XXXYY* ♂ (8 ♀♀ : 9 ♀♂ : 15 ♂♂). The autosome numbers were counted on 10 plants, only, in this sowing (S. 536). The numbers varied between 41 and 45 chromosomes. The sex expression of the intersexual plants showed the same variation as in the above sowing. The offspring of one of the intersexual plants with a small chromosome fragment in addition to the normal *XXXY* sex chromosomes were followed during one generation, and will be described below.

5. *Second generation of aneuploid intersexes.* — Seven out of the intersexual plants in S. 475 (Table 5) were selfed and the offspring are

TABLE 6. *The relation between sex chromosomes and sex in the progeny of seven aneuploid intersexes (I—VII) from sowing 475 (Table 5). The euploid sex chromosome combinations are underlined.*

Sex	Sex chromosomes	I *XXXY*	II *XXXY*	III *XXYY*	IV *XXXY*	V *XXXY*	VI *XXXY*	VII *XXXY*	Total	
♀♀	*XXX*	2	2		1		1		6	58
	XXXX	5	4	8	5	2	15	13	52	
♀♂	*XXY*			1					1	82
	XXXY	2	7	13	2	6	27	11	68	
	XXXXY					3	1	3	7	
	XXYY					1		1	2	
	XXXYY		2	1					3	
	XXXXYY	1							1	
♂♂	*XXY*	2	1	1	2				6	69
	XXXY	1	5	6	5	1	4	8	30	
	XXYY		8	4	3	2	6	5	28	
	XXXYY	1	2				1		4	
	XXYYY			1					1	
	Total	15	31	34	18	14	55	42		209

TABLE 7. *The autosome variation in the progeny of the seven aneuploid plants in Table 6.*

	Autosomes												Total
	35	36	37	38	39	40	41	42	43	44	45	46	
I							1		1	3		1	6
II						1		2	3	1	2		9
III				1	1	2	4	4	4				16
IV								1	3	2	1	1	8
V					1	1		1	1	1			5
VI						3	4	4	5	2			18
VII	1			1	5	11	7	12	3	1	1		42
Total	1	—	—	2	7	18	16	24	20	10	4	2	104

shown in Tables 6 and 7. Six plants had the sex chromosome constitution *XXXY* and were selected among the most intermediary intersexual types, the last plant was an *XXYY* androhermaphrodite. The *XXYY* plant is expected by selfing to breed almost true as to sex chromosome constitution because it forms a great excess of *XY* gametes, although a small number of *XXXX*, *XXXY* and *XYYY* plants may also

be formed owing to the production of *XX* and *YY* gametes. This assumption is not in conformity with the actual findings (Table 6); but the reason for this discrepancy remains rather obscure. The 104 autosome countings in Table 7 comprise all 58 females and specimens of males and intersexes. They show that the triploid *Melandrium* progeny tend to become stabilized on the tetraploid chromosome level, and not on the diploid level, as is usually the case in other triploid progenies.

As to the variation in sex expression the result is the same as in the previous generation. All plants give rise to females with no *Y* chromosomes, and to males and hermaphrodites with at least one *Y* chromosome. The intersexes for the greater part are again androhermaphrodites; several plants were, however, classified as almost intermediary euhermaphrodites resembling those which originated by fragmentation of the *Y* chromosome. *Hence, in the course of two generations, it has been possible by selection to produce bisexual plants having their female organs much better developed than had been the case in the first generation plants.*

One more aneuploid sowing remains to be described. Among the intersexes from sowing 536 (progeny of S. 441—1), one plant, S. 536—21, had a tiny centric fragment ($2n = 42 + XXXY + f$). The offspring of this plant are given in Table 8. Evidently, presence or absence of the fragment has no influence upon the sex expression. The autosome numbers varied from 37 to 46, but the majority of the plants had 42, 43 or 44 autosomes.

TABLE 8. *S. 699, offspring of S. 536—21, $2n = 42 + XXXY + f$.*

Sex	Sex chromosomes	No. of fragments 0	1	2	3	Total	
♀♀	*XXX*	2	1	1		4	10
	XXXX	4		2		6	
♀♂	*XXY*		1			1	9
	XXXY	3	4			7	
	XXXYY	1				1	
♂♂	*XXY*	2	1			3	17
	XXXY	5	3	1	1	10	
	XXYY	1		1		2	
	XXXYY	1		1		2	
	Total	19	10	6	1		36

Ten out of the intersexual plants grouped in Table 6 were selfed and their progeny were followed for one or two more generations. Un-

fortunately, the cytology of these plants could not be followed with sufficient care during the last years of the war; nevertheless, the rather scanty observations are in full agreement with previous observations: All the intersexual plants so far investigated had at least one *Y* chromosome and gave rise to females, males and hermaphrodites showing various degrees of intersexuality. Likewise, no female plant studied ever contained a *Y* chromosome or even fragments of a *Y* chromosome.

6. *Observations on meiosis in the aneuploid plants.* — A thorough analysis of the autosome configurations in some of the aneuploid plants was planned, but, owing to the war, this scheme could not be carried out. Some of the intersexes of the second generation were crossed to diploids in order to facilitate the analysis, but, even here, the observations are very insufficient. During the routine work of analysing the sex chromosome configurations in P.M.C.'s of the males and hermaphrodites, multivalent autosome configurations (trisomes, tetrasomes and pentasomes) were often observed together with a varying number of univalents. The configurations in a few cells which were completely analysed are grouped in Table 9. The first 5 cells derive from crossings between diploids and aneuploid intersexes, the last plant is from a second generation intersexual offspring.

TABLE 9. *Autosome associations in metaphase I, sex chromosomes not included.*

Chromosome number	Univalents	Bivalents	Trivalents	Quadrivalents	Pentavalents
33	1		9		1
31	6	5	5		
31	3	2	8		
34	3	3	7	1	
32	3	4	7		
41	1	3	3	5	1

The occurrence of pentavalents in the offspring of triploids and of tetravalents and pentavalents in crossings between diploids and aneuploids proves that non-disjunction occasionally takes place in the autosomes of the triploid offspring plants. Although statistical evidence is lacking, the impression is that the phenomenon is fairly common.

III. CONCLUSIONS.

From the present material the following conclusions may be drawn:

(1) The *Y* chromosome is male determining, because anthers never

develop in the absence of *Y*. This agrees with all previous findings from analyses on sex determination in polyploid *Melandrium*.

(2) The *X* chromosome is female determining, as appears from Table 10. This table summarizes the relation between chromosome constitution and sex in the males and hermaphrodites of the whole material.

TABLE 10. *Chromosome constitution and sex in the aneuploid Melandrium males and intersexes.*

Sex chromosome constitution	Number of *X* chromosomes	♂♂	♀♀	Total	% ♂♂
XY	1	15	0	15	100
XXY		25	3	28	
XXYY	2	37	5	42	89
XXYYY		1	0	1	
XXXY		72	133	205	
XXXYY	3	7	5	12	36
XXXXY		0	8	8	
XXXXYY	4	0	1	1	0
Total		157	155	312	

All the plants with one *X* chromosome are males; among the plants with two *X* chromosomes, 63 (89 %) are males, but in the third group with three *X* chromosomes only 79 out of 217 plants (36 %) are males, and all the *XXXX* plants are hermaphrodites. The female determining influence of the *X* chromosomes also explains the fact that no intersexual plants resulted from the crossings between diploids and triploids (Tables 2 and 3), because only *XY* and *XXY* types will appear from these combinations. From the cross 4n × 3n, however (Table 4), *XXXY* plants are formed, some of which are intersexual. With respect to the significance of the *X* chromosome, the observations are thus in full agreement with WARMKE's conclusions (WARMKE, 1946). In the Danish *Melandrium* material hitherto published, the rôle of the *X* chromosome could not be analysed, however, because in contradistinction to WARMKE's plants even tetraploid *XXXXY* plants were males.

(3) In some combinations the autosomes may act as female determiners. This fact also appears from Table 10 and from the previous tables. The difference in sex expression within a group with the same sex chromosome constitution, for instance, either within the

XXY or *XXYY* or *XXXY* or *XXXYY* groups, must be due to different autosome combinations, those in the intersexes pulling more strongly in the female direction than those in the males.

In the progeny of a triploid *Melandrium*, three combinations are possible for each of the 11 autosomes, viz. 1 *aa* : 2 *aaa* : 1 *aaaa*. Generally, only the triplex *aaa* type will segregate in the next generation, whereas the duplex and quadruplex combinations will breed true. However, non-disjunction being possible, and probably rather common (cf. Section II: 6), some autosomes may be present in the *aaaaa* or even higher combinations in the following generations.

Autosome sets with more female tendencies may *a priori* be expected to arise from such combinations in two different ways:

(1) All or most of the autosomes are slightly female determining and an accumulation of female determining autosomes together with a proper number of *X* chromosomes can overcome the influence of the male determining *Y* chromosome. (The conceptions male and female determining genes and chromosomes are used quite arbitrarily in this discussion, the proper definition of these terms being explained later in this paper.)

(2) The second alternative is that some of the autosomes are male determining and others are female determining, and the more strongly female determining combinations are due to a change in the proportion between the male and female determining autosomes. Such a change should be possible if most male determining autosomes were present in the duplex *aa* combination and most female determining autosomes in the quadruplex *aaaa*, and some even in the *aaaaa* or higher combinations in the aneuploid intersexes.

It is difficult to prove which of these two possibilities plays the more important rôle, but the second one seems to be by far the more probable. The following facts should be kept in mind:

(1) The intersexual plants recorded in Tables 1 and 4 appear among the plants with most autosomes. Moreover, the segregation of more intermediary intersexes (with better developed female organs) in the following generations is correlated to an increase in the number of autosomes, which tends towards a stabilization on the tetraploid level. This points to the significance of an accumulation of female determining autosomes in the intersexual plants.

(2) On the other hand, no intersexual plants appear in euploid series with an increasing number of autosome sets in relation to the sex chromosomes (for instance, 2 *A* + *XXY*; 3 *A* + *XXY*; 4 A + *XXY*).

This has been clearly demonstrated by WARMKE (1946), whose results are supported by the Danish material. Hence, a mere increase in the number of autosomes is not sufficient to induce intersexual development.

(3) The evidence of the existence of male determining genes in the autosomes of *Melandrium* has recently been summarized (WESTERGAARD, 1946). In two cases, intersexual plants have appeared in the euploid *Melandrium* strains in which one or two autosomes were lacking, one was a triploid $3\,A + XXY - 2\,a$ plant, the other a tetraploid $4\,A + XXXY - a$ plant (WESTERGAARD, 1940, p. 68). Accordingly, the absence of certain male determining autosomes makes the development of female organs possible (although the possibility exists, of course, that the loss of the autosomes in these plants is independent of the change in sex expression). *Hence, the appearance of the aneuploid Melandrium intersexes is most likely due to a change in the internal balance of the autosome set, altering the ratio between male and female determining autosomes, which, together with a proper number of X chromosomes, can overcome the influence of Y.*

IV. DISCUSSION.

1. *Differences between the European and the American Melandrium strains.* — The investigations into the sex mechanism of polyploid *Melandrium album* have been carried out independently in the U. S. A. by WARMKE and BLAKESLEE (cf. WARMKE, 1946), in Denmark by the present author (WESTERGAARD, 1940, 1946), and in Japan (according to preliminary reports of ONO, 1940). Although the main conclusions of the above authors are in agreement, some very significant differences seem to be present between the Danish and the American material; consequently, different conclusions concerning the relative significance of sex chromosomes and autosomes have been drawn by WARMKE and the present writer. Our material seems to be different in three respects:

(1) The *X* chromosome is more strongly female determining in the American plants. This appears from the fact that 4n *XXXXY* plants are intersexual in WARMKE's material, whereas the Danish plants are males.

(2) The three different sections in the *Y* chromosome (cf. Fig. 2) have been demonstrated by means of spontaneous deficiencies in both materials. However, in the Danish plants the suppressor region (cf. Fig. 2, section I) is located in the differential arm and the »fertility genes» (Fig. 2, section III) in the section between the centromere and

the homologous section (WESTERGAARD, 1946), while the position of the two complexes seems to be reverted in the American material (preliminary reports by WARMKE, 1946 a). This difference may be best explained on the assumption that a centric inversion in the *Y* chromosome has changed the positions of the two sections. This structural re-arrangement in the *Y* chromosome is rather interesting, because *Melandrium* is not a native species of America, but was first introduced with the white man. A study of the geographical distribution of the two types of *Y* chromosomes might show whether the re-arrangement has occurred after the plant was introduced into the U. S. A., or whether

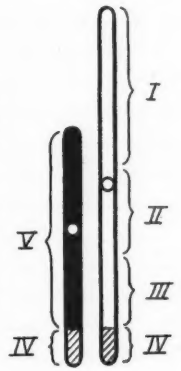


Fig. 2. Diagram of the *Melandrium* sex chromosomes. — I: Female suppressor region in *Y*; when lost, a bisexual plant appears. — II: Essential male promotor region; when absent, a female plant results. — III: Essential male fertility region; when lost, male sterile plants with abortive anthers appear. — I + II + III: Differential parts of *Y*. — IV: Pairing region. — V: Differential part of *X*. — [Since the loss of regions III + IV (the Y^3 type described by WESTERGAARD, 1946) results in male sterile plants, the fertility genes must be in the differential part of *Y*, if not, the effect of the loss would be compensated by the genes in the *X* chromosome. Therefore, the pairing segments in *X* and *Y* must be shorter than assumed in the previous publication, but the length proposed in Fig. 2 is still somewhat arbitrary.]

both types are found in Europe. A crossing between the two strains would likewise be very interesting.

(3) No hermaphrodites appeared in the progeny of triploids in the American material (WARMKE and BLAKESLEE, 1940), whereas a fairly large number of the Danish plants exhibited various degrees of intersexuality. This is probably due to a different internal balance between the male and female determining autosomes in the autosome sets of the two samples. In this connection, it should be remembered that the plants studied in Denmark derived from *Melandrium* seeds secured from the Botanical Gardens in Germany, Roumania and Russia (WESTERGAARD, 1940) whereas the American plants derived from different localities in the United States (WARMKE and BLAKESLEE, 1940). Therefore, the present material may initially have been more heterogeneous than that of the American authors.

2. *The theory of sex determination.* — The work on sex determination in diploid and polyploid *Melandrium* shows most strikingly

the existence of two different complexes in sex determination and their localization in autosomes and sex chromosomes. The one complex is composed of the sex promoting genes or basic sex genes for the development of the male and female organs and for the whole normal sexual pattern of the organism. These genes are probably located in all the chromosomes (except that female promoting genes cannot occur in the differential segment of the *Y* chromosome) and, consequently, are present in both sexes. The sex promoting genes interact with another complex: A trigger mechanism, the sex deciding genes which switch the development into a male or a female, whereas the sex promoting genes alone would make a bisexual organism. The trigger complex is confined to the differential segments of the sex chromosomes.

At the present time, the mechanism of the male trigger only in *Melandrium* is more thoroughly known. Maleness is maintained by means of the female suppressor in the differential arm of *Y*. If the suppressor is lost, a bisexual plant appears; hence, the only function of this trigger is to suppress the female organs. *The trigger is not a part of the male promoting system* (cf. WESTERGAARD, 1946 and Fig. 2).

The mechanism of the maintenance of femaleness in *Melandrium* is not as well understood as that of the male trigger. The existence of female determining genes in the differential part of *X* (WARMKE, 1946) does not necessarily mean that this segment contains the female sex deciding genes. There is, however, some indirect evidence for the existence of a male suppressor in this segment, which inhibits the effect of the male determining genes in the autosomes, but is hypostatic to the male determining *Y* chromosome (WESTERGAARD, 1946). *Nevertheless, it is much more probable that the Y chromosome also acts as the trigger chromosome for femaleness.* The analysis of the fragmented *Y* chromosomes has shown that, besides the female suppressor (section I, Fig. 2), at least two different sections in the differential part contain male promoting genes: If the sections denoted by III and IV (Fig. 2) are lost, the anthers degenerate shortly after meiosis, and male sterile plants appear. If the whole chromosome is absent (in females), anthers never develop; therefore, section II, comprising the centromere region, must contain genes which control an essential step (or steps) in promoting the anthers. *The trigger mechanism in Melandrium is built up by means of an absolute linkage between the female suppressor and some essential male promoting genes, all of which are in the differential segment of the Y chromosome. The presence of the Y chromosome determines maleness instead of bisexual development by suppress-*

ing some essential step or steps in the development of the female organs, and the absence of the Y chromosome determines femaleness instead of intersexual development because some essential steps in the development of the male organs are blocked.

The sex promoting mechanism is, of course, the original system, present also in the bisexual ancestors of Melandrium and in related bisexual species, whereas the trigger mechanism originated with the evolution of dioecism.

This distinction between two mechanisms in sex determination is of great significance. It is also a very old conception in sex genetics, advocated by MULLER as early as in 1916 (cf. MULLER, 1932). The quantitative interaction between trigger chromosome and autosomes was demonstrated in *Drosophila* by BRIDGES (1939) and the existence of both male and female promoters in the autosomes is proved in an especially clearcut manner in the work on sex reversal in *Lebistes* by WINGE (1934). The same dualistic principle is the fundamental element in the conceptions of sex potency and sex realisators of the German geneticists (CORRENS, HARTMANN). As will be discussed below, however, the terminology and the symbols introduced by the German school sometimes obliterated this distinction. Besides, a theory of sex determination must embrace the genetical as well as the cytological mechanism, and the credit for having explained the sex determination mechanism in relation to the general theories of crossing-over and chiasmatypy must be given to DARLINGTON who, by introducing the terms pairing and differential segments in the sex chromosomes, and the conception of permanent structural hybridity, defined the problem much more trenchantly than had been done before (cf. DARLINGTON, 1932).

In the above discussion the term sex determining genes has been used in a quite neutral sense comprising both the sex deciding genes (or trigger genes; both terms have been suggested by MULLER) and the sex promoting genes (the term has been used by JONES, 1934, in the work on dioecious corn) or basic sex genes. Since the discussion of the proper terminology and the symbols in sex genetics still plays an important part in the literature (for reference, see HÄMMERLING, 1937; HARTMANN, 1943; KNAPP, 1943), it seems justified to examine the various theories and terminologies in relation to the *Melandrium* experiments.

CORRENS (1928) used the *AGZ* complexes and the α and β realisators as symbols in sex genetics. In these formulae, *A* stands for the genes which cause the development of the male sexual organs and *G* for the

development of the female organs, whereas *Z* is a »timing complex» which controls the time of development of the sexual organs. α and β are the realisators for maleness and femaleness, present in dioecious organisms only. The *AGZ* complex would, thus, correspond to the sex promoters in the autosomes of *Melandrium*, whereas α and β would be the sex deciding genes. As to the justification of using special symbols in sex genetics, it should always be kept in mind that the study of this problem is by no means different from the study of other genetical phenomena. However, it was always a good rule in genetics to introduce special symbols only for genes which could be studied in at least two allelic forms for the very good reason that only such genes can be handled by the ordinary genetical methods. There is no reason whatsoever to abandon this rule in sex genetics, and the use of CORRENS's symbols as well as of other special symbols should therefore be given up, because in sex genetics we are dealing with a polygenic system in which the function of the individual genes is generally unknown. The term sex deciding genes or trigger genes is preferred to the term realisator genes because it does not seem adequate to call the female suppressor in *Melandrium*, which does not participate in the promotion of the male organs, a realisator of maleness.

HARTMANN (1943) has abandoned the *Z* complex of CORRENS, but maintains the *A* and *G* complexes. Moreover, he uses *M* and *F* instead of α and β for the realisators which, according to HARTMANN, must be present even in bisexual organisms, where they counterbalance each other. As to the use of the special symbols, the criticism which has been expressed above of course applies to these formulae also. Moreover, I do not see any reason to assume the existence of realisators in bisexual organisms. This assumption is unlikely from an evolutionary standpoint and, with a trigger mechanism like the one in *Melandrium*, it would even be impossible. HARTMANN summarizes the principles of sex determination in three laws (HARTMANN, 1943, p. 360): (1) »Das Gesetz der allgemeinen bipolaren Zweigeschlechtlichkeit», (2) »das Gesetz der allgemeinen bisexuellen Potenz», and (3) »das Gesetz der relativen Stärke der männlichen und weiblichen Determinierung».

HARTMANN's second law is in full agreement with the *Melandrium* work, which shows the existence of both male and female promoters in the autosomes. The term »sex promoters» or »basic sex» genes is preferred to the term »sex potencies» as more adequate to the purpose. However, the significance of the distinction between HARTMANN's first and second law is not clear, and HARTMANN himself admits that the

distinction is a rather vague one. He writes (1943, p. 361): »Immerhin erscheint es angebracht, künftig begrifflich die allgemeine bisexuelle Potenz in weiterer unbestimmter Fassung (HARTMANN) von dem bisexuellen, genetisch gekennzeichneten A.G.-Komplex (CORRENS) zu unterscheiden. Es besteht zwar die Möglichkeit, ja die Wahrscheinlichkeit, dass diese allgemeine bisexuelle Potenz künftig in ein System von A.G.-Erbfaktoren weiter auflösbar ist . . .».

At least in the study of sex genetics in the higher plants and animals, the distinction between the first and second law is insignificant. The question is still open whether the distinction applies to the algae and fungi in which heterothallism is often maintained either by a sexual differentiation analogous to the mechanism in the dioecious plants and animals, or by an incompatibility mechanism analogous to the »*S*» mechanism in the higher plants, or by both mechanisms. HARTMANN's third law on the relative strength of the male and female determiners is linked to his theory on the existence of sex realisators in bisexual organisms also. It is probably, as was CORRENS's Z complex, defined under the influence of GOLDSCHMIDT's *Lymantria* work and his »turning point» conception. It should be admitted that, whenever the sex mechanism of an organism has been more thoroughly analysed, it was possible to show a quantitative interaction between the sex determining genes, but in most cases this has been an interaction between the sex deciding genes and the sex promoting genes. Thus, in most organisms, first and foremost in the bisexual plants, the theory of the relative strength of the sex determining genes remains a postulate. It should also be pointed out that GOLDSCHMIDT's »turning point» theory as a general principle in the mechanism of sex determination is a postulate, and recent embryological and histological investigations on intersexual *Drosophila* (NEWBY, 1942; cf. STONE, 1942) and *Solenobia* (SEILER, 1945) have not supported the theory (see also BALTZER, 1937 and BRIDGES, 1939).

The general principles of sex determination may be summarized as follows: *The sex of a dioecious organism is determined by a quantitative interaction between sex promoting genes (basic sex genes) for the development of the male and female sexual organs and sex deciding genes (the trigger mechanism) which switch the development into a male or a female. The sex promoting genes are distributed to all chromosomes (except that female promoting genes cannot occur in the differential part of the Y chromosome) and are therefore present in both sexes, whereas the sex deciding genes are restricted to the differential part of the sex chro-*

mosomes (or sex chromosome). The sex chromosomes may or may not be morphologically differentiated. The progress in sex genetics depends upon to what extent it will be possible to analyse the morphological, physiological and developmental functions of the individual genes which take part in this mechanism. It will always be a matter of convenience which genes should be called sex determining, because it will depend upon which aspect of the sex problem is under investigation and upon how far it is possible to understand the function of the individual genes.

In recent literature, the *Drosophila* and *Melandrium* mechanisms of sex determination are often referred to as the »*Drosophila* type» and »*Melandrium* type». Attention should be called to the fact that these »types» are not clearly defined. Some authors speak of the »*Drosophila* type» as the mechanism where sex is determined by the interaction between an *X* trigger chromosome against the autosomes with an almost inert *Y* chromosome, and »*Melandrium*» as the »type» with a male determining *Y* chromosome. Other authors seem to think of the »*Drosophila* type» as the special system of balance where tetraploid *XXXY* individuals are intersexes, whereas »*Melandrium*» represents the type of balance where such individuals are males. The first balance blocks the establishment of a tetraploid dioecious race, but the *Melandrium* balance makes the establishment of such a race possible. There is no reason to believe that the interaction between the sex determining genes depends upon whether the *Y* chromosome or the *X* chromosome is the trigger chromosome, and future work will undoubtedly reveal organisms in which the *Y* chromosome is male determining (as in the case of *Melandrium*), but where tetraploid *XXXY* individuals are intersexes (as in *Drosophila*), and *vice versa* (cf. KNAPP, 1943). The genetics of sex determination have always suffered from the fact that a more thorough understanding of the mechanism was only possible in very few species and too far-reaching generalizations have been drawn from these isolated cases. The establishment of too many »types» in sex determination is therefore likely to do more harm than good.

3. *Evolution of the trigger mechanism.* — It should be emphasized that there is no reason to believe that the trigger mechanism always acts through a direct suppression of the sex organs of the opposite sex, as in the case of *Melandrium*. In *Drosophila*, for instance, the trigger mechanism is in the differential part of the *X* chromosome, and there is no basis for assuming that it is the function of these genes to suppress the development of the male organs in the female. It is more likely that the trigger is due to an accumulation of female promoting genes

distributed all over the differential segment (DOBZHANSKY and SCHULTZ, 1934; PIPKIN, 1940; cf. STONE, 1942) which interacts with the male determining genes in the autosomes.

Undoubtedly, *Drosophila* can be regarded as a much more advanced type of dioecious evolution, whereas *Melandrium* represents a more recent evolution of dioecism from not very remote bisexual ancestors. In the primitive state, the evolution of a dioecious organism from a bisexual one by means of a suppressor mechanism seems to be a very logical step. This is also the mechanism in dioecious corn, which represents the most primitive example of the origin of dioecious strains from bisexual ancestors (EMERSON, 1932; JONES, 1934). The »silkless» gene in corn may be regarded as a female suppressor and the »tassel seed» gene as a male suppressor. The sex of the plant is the result of a quantitative interaction between the two triggers, because the »silkless» gene is hypostatic to the homozygous »tassel seed» condition, but not to the heterozygous »tassel seed» combination. The experimentally synthesized dioecious corn strains show a trigger mechanism *in statu nascendi*. In this stage, the maintenance of dioecism seems to require two triggers, one which makes a male out of a bisexual plant and one which converts a bisexual plant into a female.

The further evolution of dioecism will probably, to a large extent, be influenced by the special type of selection to which the genes in the potential *Y* chromosome are exposed. As stressed by MULLER (1932) and DARLINGTON (1932), a stabilization of the sex deciding mechanism requires a suppression of crossing-over in the section of the chromosomes which carries the trigger. Since the genes in the differential segment of the *Y* chromosome can never be present in the female individuals, only such genes can be *Y* linked as have no selective significance for the vegetative development of the individual and for the development of the female sex characters. The evolution of the differential part of the *Y* chromosome will, therefore, depend upon to what extent the function of the genes in the potential *Y* chromosome, which are not directly connected with the trigger mechanism, can be taken over by autosomal genes. Moreover, genes in the differential part of the *Y* chromosome which have no selective significance for the individual will gradually be lost by mutations, as the organism has no way of compensating these losses when crossing-over is suppressed. Consequently, only such genes will persist in the differential part of *Y* (true *Y* linked genes) as are part of the trigger mechanism or genes for the development of the primary and secondary male characters [e. g.,

the fertility genes in the *Y* chromosome of *Drosophila* (STERN, 1929), the sex limited colour genes in *Lebistes* (WINGE, 1927 *et al.*), and the male promoting genes in *Melandrium*], whereas the rest of the differential segment in *Y* will become inert. *In Melandrium the development of the female suppressor (by mutation) has probably been the first step towards dioecism, and the suppression of crossing-over in the part of the chromosome which carried the mutation has established that absolute linkage between the mutation and some essential male promoting genes which is the present trigger mechanism. At the same time, the rest of the differential Y chromosome has become inert.*

However, a sex deciding mechanism in which the *Y* chromosome is the actual trigger chromosome may represent a rather unstable system in diploid organisms, where the *Y* chromosome will be the only »constant haploid chromosome», because such a mechanism may be rather susceptible to gene mutations and chromosome breaks. An establishment of a new trigger mechanism in the course of time may, therefore, be favoured by natural selection. This would be a mechanism in which the *X* chromosome is the only trigger chromosome. A development of such a mechanism would require the function of the male determining genes in the *Y* chromosome to be taken over by the autosomes and it would require the establishment of a new level of balance between the *X* chromosomes and the autosomes. This may be the situation in *Drosophila*, where the trigger mechanism obviously has a long evolutionary history. In this case the secondary trigger mechanism has been established by accumulation of female determining genes, probably female promoters in *X*. In this connection it seems to be very significant that recent studies on sex determination in *Drosophila* have shown the presence of female suppressor genes in the autosomes (LEBEDEFF, 1939; STONE, 1942; STURTEVANT, 1945). These may very well represent the rudiments of an original *Y* linked trigger mechanism, in which the developmental function of the individual genes has been taken over by the autosomes and the trigger function of the gene complex has been substituted by the present *X* mechanism.

The above suggestions may be a much too schematic and dogmatic working hypothesis for one of the possible modes of evolution of a trigger mechanism from *Zea* via *Melandrium* to *Drosophila*. The scheme might be further elaborated or disproved if the gap between maize and *Melandrium* and between *Melandrium* and *Drosophila* could be bridged by the study of sex genetics in other dioecious organisms. The dioecious plant species may offer favourable opportunities for studying the differ-

ent ways in which dioecism has come into being, dioecism having undoubtedly arisen independently in the different plant genera. However, as already stated above, future progress in sex genetics will depend upon our ability to interpret the function of the individual genes in terms of biochemical and physiological processes. For this line of research the higher plants and animals may be a less suited material than the lower organisms, which have already given important results in the hands of HARTMANN and his co-workers (for references, see HARTMANN, 1943) and in the work on sex hormones in *Achlya* by RAPER (literature in RAPER, 1942).

Acknowledgements. — The author wishes to express his gratitude to the Carlsberg Foundation, which has supported these *Melandrium* investigations with a grant. My best thanks are further due to Professor C. A. JØRGENSEN, Ph. D., Chairman of the Department of Genetics, for excellent facilities and for helpful criticism, and to Mr. OTTO MATHIESEN, in charge of the experimental field of the Department. Finally, I am indebted to Mr. SAI-CHIU VAN, M. Sc., Mr. ALFRED ANDERSEN, M. Sc., and Miss. L. CHRISTOPHERSEN, B. Sc., for technical assistance in the preparation of the microscopical slides.

SUMMARY.

Intersexual plants (androhermaphrodites) appear in the progeny of triploid *Melandrium* crossings, and from crossings between triploids and tetraploids, but not from crossings between triploids and diploids.

The progeny of some intersexual plants have been followed genetically and cytologically through several generations. The intersexual plants give rise to females without a *Y* chromosome and males or intersexes with at least one *Y* chromosome.

The following conclusions concerning the mechanism of sex determination in *Melandrium* have been drawn: (1) The *Y* chromosome is male determining. (2) The *X* chromosome is female determining. (3) Some of the autosomes are female determining, others are male determining. The appearance of the aneuploid intersexes in the progeny of triploids is explained as due to a change in the internal balance of the autosome complement, resulting in an altered ratio between male and female determining autosomes which, in combination with a proper number of *X* chromosomes, overcome the influence of the male determining *Y* chromosome.

The work on sex determination in *Melandrium* has been sum-

marized, and the sex mechanism is explained as follows. Sex is determined by a quantitative interaction between two gene complexes: (1) The male and female promoting genes located in all the chromosomes and present in both sexes, and (2) the sex deciding genes, a trigger mechanism restricted to the differential part of the *Y* chromosome. The presence of *Y* determines maleness instead of bisexual development by suppressing the female organs, and the absence of *Y* determines femaleness because some essential steps in the development of the male organs are blocked. The efficiency of the trigger mechanism is due to the absolute linkage between the female suppressor complex and some essential male promoting genes in the *Y* chromosome.

The *Melandrium* experiments have been critically discussed in relation to the current theories of sex determination. It is suggested that the use of special symbols and formulae in sex genetics (CORRENS, HARTMANN) should be abandoned. HARTMANN's three laws on sex determination have been critically analysed and an alternative formulation of the general principles of sex determination is suggested.

The trigger mechanism in dioecious *Zea*, *Melandrium* and *Drosophila* has been discussed in relation to the problem of the evolution of dioecism.

The genetical differences between the American *Melandrium* strains studied by WARMKE and the European strains studied by the present author are pointed out.

LITERATURE CITED.

1. BALTZER, F. 1937. Analyse des GOLDSCHMIDTschen Zeitgesetzes auf Grund der *Bonellia*- und *Lymantria*-Intersexe. — W. R. Arch. Entw. Org. 136, 1—87.
2. BRIDGES, C. B. 1939. Cytological and genetical basis of sex. — In ALLEN: Sex and internal secretion, 2nd Ed., 15—63.
3. CORRENS, C. 1928. Bestimmung, Verteilung und Vererbung des Geschlechtes bei den höheren Pflanzen. — Handb. d. Vererbungswiss. 2, 1—138.
4. DARLINGTON, C. D. 1932. Recent advances in cytology. 1st Ed. — London.
5. DOBZHANSKY, TH. and SCHULTZ, J. 1934. The distribution of sex factors in the *X* chromosome of *Drosophila melanogaster*. — Journ. of Gen. 28, 349—86.
6. EMERSON, R. A. 1932. The present status of maize genetics. — Proc. 6th Internat. Congr. Genetics 1, 141—67.
7. HARTMANN, M. 1943. Die Sexualität. — Jena.
8. HÄMMERLING, J. 1937. Fortpflanzung und Sexualität. — Fortschr. d. Zoologie. N. F. 1, 384—406.
9. JONES, D. F. 1934. Unisexual maize plants and their bearing on sex differentiation in other plants and animals. — Genetics 19, 552—67.

10. KNAPP, E. 1943. Bemerkungen zu Geschlechtsbestimmungsfragen. — Flora N. F. 37, 139—51.
11. LEBEDEFF, G. A. 1939. A study of intersexuality in *Drosophila virilis*. — Genetics 24, 553—86.
12. LÖVE, D. 1944. Cytogenetic studies on dioecious *Melandrium*. — Botaniska Notiser, Lund 1944, 125—213.
13. MULLER, H. J. 1932. Some genetic aspects of sex. — Amer. Nat. 66, 118—37.
14. NEWBY, W. W. 1942. A study on intersexes produced by a dominant mutation in *Drosophila virilis*, Blanco stock. — Univ. Texas Publ. 4228, 113—46.
15. ONO, T. 1939. Polyploidy and sex determination in *Melandrium*. — Bot. Magazine, Tokyo 53, 550—56.
16. PIPKIN, S. B. 1940. Multiple sex genes in the *X* chromosome of *Drosophila melanogaster*. — Univ. Texas Publ. 4032, 126—57.
17. RAPER, J. R. 1942. Sexual hormones in *Achlya*. III. — Amer. Journ. of Bot. 29, 159—66.
18. SEILER, J. 1945. Analyse eines intersexen Freilandtieres von *Solenobia alpicolella* R. — Arch. Julius Klaus-Stiftung 20, 217—35.
19. STERN, C. 1929. Untersuchungen über Aberrationen des *Y*-Chromosoms von *Drosophila melanogaster*. — Zschr. f. ind. Abst.- u. Vererbl. 51, 253—352.
20. STONE, W. S. 1942. The I_x^B factor and sex determination. — Univ. Texas Publ. 4228, 146—52.
21. STURTEVANT, A H. 1945. A gene in *Drosophila melanogaster* that transforms females into males. — Genetics 30, 297—99.
22. WARMKE, H. E. 1946 a. An analysis of male development in *Melandrium* by means of *Y* chromosome deficiencies. — Genetics 31, 234.
23. — 1946 b. Sex determination and sex balance in *Melandrium*. — Amer. Journ. of Bot. 33, 648—60.
24. WARMKE, H. E. and BLAKESLEE, A. F. 1940. The establishment of a 4n dioecious race in *Melandrium*. — Amer. Journ. of Bot. 27, 751—62.
25. WESTERGAARD, M. 1940. Studies on cytology and sex determination in polyploid forms of *Melandrium album*. — Dansk Bot. Ark. 10, 1—131.
26. — 1946. Aberrant *Y* chromosomes and sex expression in *Melandrium album*. — Hereditas XXXII, 419—43.
27. WINGE, Ø. 1927. The location of eighteen genes in *Lebistes reticulatus*. — Journ. of Gen. 18, 1—43.
28. — 1934. The experimental alteration of sex chromosomes into autosomes and vice versa as illustrated by *Lebistes*. — Compt. Rend. Trav. Lab. Carlsberg 21, 1—49.

LINKAGE VALUES IN AN INTERCHANGE COMPLEX IN PISUM

BY *ROBERT LAMM*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

INTRODUCTION.

IN a recently published paper some genetical data are given concerning a case of reciprocal translocation in *Pisum sativum* (LAMPRECHT, 1946). These data refer to the progenies of selfed translocation heterozygotes. In a lecture before the Mendelian Society, Lund, in the autumn of 1944 E. NILSSON gave a preliminary report of the investigations made by him on probably the same translocation. He had, however, used the backcross method. From his as yet unfinished and hence unpublished investigations he has kindly permitted me to quote some data, which will here be compared with those given by LAMPRECHT. The latter data will be more exhaustively calculated and discussed in this paper.

SURVEY OF THE INVESTIGATIONS.

The reciprocal translocation studied by LAMPRECHT (1946) was investigated in his cross No. 353 between the pure line 232 (A Uni m mp f Fs st b) of standard constitution and the line 379 (a Uni m mp f fs St B) of deviating structural type. The F_1 was semisterile with about 50 % seed abortion. Segregation of the genes Fs, St and B was studied. The first of these genes belongs to a linkage group which according to LAMPRECHT (1942 a) probably has the constitution Gp—3,2 %—Cp—32,2 %—Fs—$<$1 %—Ast, whereas the second and third gene belong to the group Uni—4 %—M—26 %—Mp—about 15 %—F—14 %—St—26 %—B—16 %—Gl (LAMPRECHT, 1946). These linkage relations represent what will be called the Gp- and B-chromosomes of standard types. It is perhaps worthy of remark that the existence of the gene F in standard lines ought to be more conclusively proved, but as this question does not come within the scope of the present paper it will not be further discussed here.

Line 379 originated from the cross Weibulls Ambrosia I $\times$ Extra Rapid. The last-mentioned variety is probably responsible for the

translocation, which seems to be identical with that originally found by HAMMARLUND in the cross Extra Rapid × Soloärt (HÅKANSSON, 1931). According to HÅKANSSON (1934), the chromosomes taking part in the Extra Rapid interchange are 5 · 6 and 7 · 8. E. NILSSON (1936) has denoted the structural type represented by Extra Rapid as »D», while SANSOME (1937) has called it »structural type 4» with chromosomes 3 and 5 of the standard type interchanged.

Data as to the origin of line 232 are given by LAMPRECHT (1942 b). According to his paper, the speckling of the seed-coat of this De Winton line is determined by the gene Fs and not by F. It is therefore astonishing that in presenting the results of cross No. 353 LAMPRECHT (1946) assumes that the gene segregating for the speckling of the seed-coat is F. The pseudo-linkage found by him between this character and the characters determined by the genes B and St does not justify the symbol F instead of Fs.

In F_2 of cross No. 353 LAMPRECHT obtained the following segregation.

412	fertile	Fs	: 268	fertile	fs	: 582	semisterile	Fs	: 70 semisterile fs
521	»	St	: 361	»	st	: 765	»	St	: 56 » st
449	»	B	: 231	»	b	: 564	»	B	: 88 » b

The segregation of St was determined on all the F_2 plants while the Fs- and B-segregation had to be restricted to individuals of the A-genotype. Crossing-over values between the genes involved have been published by LAMPRECHT (1946) and are given here in Table 3. Although no crossing-over values between the point of translocation (Tr) and the genes Fs, St and B have been computed, LAMPRECHT suggests that Tr is situated in the segment between F and St of the B-chromosome and between Fs and Ast of the Gp-chromosome.

The translocation heterozygote studied by E. NILSSON was derived by crossing a line from De Winton (st b) × Extra Rapid (St B). From his investigations I have been allowed to publish the following figures of the backcross F_1 × De Winton:

2	fertile	B	: 43	fertile	b	: 57	semisterile	B	: 16 semisterile b
1	»	St	: 44	»	st	: 61	»	St	: 12 » st

55 B St : 4 B st : 7 b St : 52 b st

E. NILSSON has made his crosses reciprocally. His results seem to be of considerable interest. They will, however, be investigated more extensively before publishing.

DETECTION OF LINKAGE.

Linkage between a gene (A) and the point of translocation (Tr) could be detected by the χ^2 method. Applying the general methods recommended by MATHER (1946, p. 183), the total χ^2 could be divided into three components, representing the segregation of fertile : semisterile, A : a and the linkage component. Assuming the progeny to be:

a fertile A : b fertile a : c semisterile A : d semisterile a,

where $a + b + c + d = n$ (the total number of plants), the χ^2 formulae below could be applied.

Progeny	selfed	backcrossed
χ^2 fertile : sterile	$\frac{(a+b-c-d)^2}{n}$	$\frac{(a+b-c-d)^2}{n}$
χ^2 A: a	$\frac{(a-3b+c-3d)^2}{3n}$	$\frac{(a-b+c-d)^2}{n}$
χ^2 linkage	$\frac{(a-3b-c+3d)^2}{3n}$	$\frac{(a-b-c+d)^2}{n}$

In Table 1 a complete χ^2 analysis has been made of the data from cross No. 353 and those from E. NILSSON's backcross.

TABLE 1. *χ^2 analysis referring to selfed and backcrossed translocation heterozygotes.*

χ^2 for	F_2 of LAMPRECHT's cross No. 353			E. NILSSON's backcross	
	Fs	St	B	St	B
fertile : sterile	0,59	2,19	0,59	6,64**	6,64**
gene segregation ..	0,10	0,24	0,78	0,31	0,00
linkage	146,07***	262,92***	74,06***	71,73***	56,63***
Total	146,76***	265,35***	75,43***	78,68***	63,27***

Table 1 shows that there is a significant deviation from the ratio 1 : 1 with respect of fertility in the backcross progeny. Hence the segregation data of this progeny are of a somewhat doubtful value if used for the estimation of linkage values comparable with those of cross No. 353.

ESTIMATION OF LINKAGE.

Let us consider the estimation of the recombination fraction p in the case of a factor A and the point of translocation Tr in an F_2 progeny

obtained from the selfing of a translocation heterozygote. Assuming that this heterozygote is of a fairly regular type in which the chromosomes involved are almost equal-armed with the centromere near the middle and in which the translocation has interchanged approximately whole arms, and further in which the separation and orientation of the chromosomes are regular and at random, the expected frequencies of the possible zygotic combinations could easily be given in terms of p. Applying the method of maximum likelihood (cf. MATHER, 1946), p could be estimated in the way shown in the theoretical example below.

Categories:	fertile A	fertile a	semisterile A	semisterile a
Nos. of plants	a	b	c	d
Proportions exp.	$\frac{1+2p-2p^2}{4}$	$\frac{1-2p+2p^2}{4}$	$\frac{2-2p+2p^2}{4}$	$\frac{2p-2p^2}{4}$

The logarithm likelihood expression is then

$$L = a \log \frac{1+2p-2p^2}{4} + b \log \frac{1-2p+2p^2}{4} + c \log \frac{2-2p+2p^2}{4} + d \log \frac{2p-2p^2}{4}$$

and, maximizing by differentiation and equating to zero, the equation for estimating the value of p becomes

$$\frac{2a}{1+2p-2p^2} - \frac{2b}{1-2p+2p^2} - \frac{c}{1-p+p^2} + \frac{d}{p-p^2} = 0$$

This equation can be solved by inserting a suitable value of p obtained by approximation and interpolation (cf. MATHER, 1946, p. 209). A first rough approximation of p could be obtained from the equations

$$\frac{a}{b} = \frac{1+2p-2p^2}{1-2p+2p^2} \quad ; \qquad \frac{c}{d} = \frac{2-2p+2p^2}{2p-2p^2};$$

$$p^2 - p + \frac{a-b}{2(a+b)} = 0. \qquad p^2 - p + \frac{d}{c+d} = 0.$$

The variance and standard error of p are obtained from the iteration solution as shown by MATHER (l. c., p. 212).

When calculating the p values of cross No. 353 I have used the above formulae, although the structure and behaviour of the ring of four in this cross may deviate considerably from the regularity postulated. This must be remembered when the results of the computations are interpreted.

In the backcross, progenies p and the standard error sp are determined by the formulae below (cf. MATHER, 1938).

$$p = \frac{a+d}{n}; \quad sp = \pm\sqrt{\frac{p(1-p)}{n}}$$

As a practical demonstration the iteration solution of p with respect to Tr—Fs of cross No. 353 is given in Table 2.

TABLE 2. *The iteration solution of the recombination fraction p between Tr and Fs of cross No. 353.*

p	0,121	0,122	0,1217	0,1218
$+\frac{2\cdot412}{1+2p-2p^2}$	+ 679,4655	+ 678,6182	+ 678,871961	+ 678,787348
$-\frac{2\cdot268}{1-2p+2p^2}$	— 680,8233	— 682,1352	— 681,741481	— 681,872699
$-\frac{582}{1-p+p^2}$	— 651,2682	— 651,8204	— 651,654802	— 651,710004
$+\frac{70}{p-p^2}$	+ 658,1483	+ 653,4971	+ 654,884300	+ 654,421138
Total	+ 5,5223	— 1,8403	— 0,359978	— 0,374217
Diff.	7,3626		0,734195	

The last line of Table 2 shows that a change of 1 would alter p by 7341,95 which is the mean value of I_p. The standard error sp is now obtained by solution of the equation

$$sp = \sqrt{\frac{1}{Ip}} = \sqrt{\frac{1}{7341,95}} = \pm 0,01167$$

TABLE 3. *Linkage relations of cross No. 353 and* E. NILSSON's *backcross.*

Linkage between	Cross No. 353 F_2	E. NILSSON's backcross [1]
Tr—Fs	12,2 ± 1,17	
Tr—St	8,0 ± 0,90	11,0 ± 2,88
Tr—B	17,5 ± 1,39	15,3 ± 3,31
B—St	12,8 ± 0,99	9,3 ± 2,68
B—Fs	23,7 ± 2,56	
Fs—St	13,5 ± 2,68	

[1] The uncertainty of these linkage data has been discussed before.

The crossing-over value between Tr and Fs is thus $12{,}2 \pm 1{,}17$ %. A survey of the estimations of linkage is given in Table 3.

INTERPRETATION AND DISCUSSION OF THE RESULTS.

The data which are summarized in Table 3 definitely place the order in the B-chromosome as Tr—St—B. The results of E. NILSSON's backcross seem to be in fairly good agreement with the corresponding results of cross No. 353, which was to be expected since both investigations probably refer to the Extra Rapid translocation. LAMPRECHT (1946) assumes that the break in the Gp-chromosome is probably situated between Fs and Ast. Since, however, the distance between these genes is less than 1 % (LAMPRECHT, 1942 a) and the distance between Tr and Fs according to Table 3 is about $12{,}2$ %, the point of translocation is not situated between Fs and Ast. Investigations designed to find the linkage between Tr and Gp are reported to be in progress. If these should show that the Gp-chromosome is really involved, the list of structural types published by SANSOME (1937) must be wrong with respect to the structural type 2 (HAMMARLUND's K-line) and the structural type 4 (Extra Rapid). According to SANSOME, those types have no chromosome interchanged in common. PELLEW (1940), however, has shown that the Gp-chromosome is involved in the interchange giving rise to the K-line. She found about 1 % of crossing over between Tr and Gp and about 20 % between Tr and Fs. Unfortunately, F_1 between the K-line and Extra Rapid has not been cytologically investigated.

In the normal or standard type there is about 26 % of crossing over between St and B. The linkage value between these genes given in Table 3 is only about $12{,}8$ %. The discrepancy between linkage values found in standard lines as compared with those estimated in translocation heterozygotes may be due to various circumstances (cf. SANSOME, 1932, p. 216). In most cases crossing over near the point of interchange is reduced. As regards the distance between St and B of the translocation heterozygote, the explanation of the much reduced crossing-over value is perhaps partly that this region of the chromosome is situated between Tr and the centromere. If this hypothesis is correct the centromere should then be located to the right of B. A renewed cytological investigation of the association of four could perhaps answer the question whether interstitial chiasmata are common or not. A comparison with the linkage values in the translocation heterozygote

between the genes of the B-chromosome to the left of Tr, e. g. the genes M and Mp, would be valuable. The genetical consequences of the occurrence of an interstitial chiasma in the region between Tr and the centromere has been discussed by my colleague at the State Horticultural Research Station Dr. G. TOMETORP in an unpublished paper. The following discussion is mainly based on that paper kindly put at my disposal by him.

In a translocation heterozygote giving a ring of four between the chromosomes $1 \cdot 2$, $2 \cdot 3$, $3 \cdot 4$ and $4 \cdot 1$ three orientations at the first metaphase of meiosis are possible, as shown below.

Zigzag orientation	Non-zigzag orientation	
A	B_1	B_2
$1 \cdot 2 \quad 4 \cdot 3$ $1 \cdot 4 \quad 2 \cdot 3$	$2 \cdot 1 - 1 \cdot 4$ $2 \cdot 3 - 3 \cdot 4$	$1 \cdot 4 - 4 \cdot 3$ $1 \cdot 2 - 2 \cdot 3$

We denote the frequencies of the orientations A, B_1 and B_2 as a, b and c ($a + b + c = 1$). Further, x is the chiasma frequency of an interstitial region between Tr and the centromere. The crossing-over value p of the gametes is then given by the formula:

$$p = \frac{b \cdot x}{2a - ax + bx}.$$

Provided that a is equal to b the value of p (quite apart from the value of c) will correspond to half the value of x:

$$a = b \quad p = \frac{x}{2}$$

This is of course the normal relation between crossing over and chiasma frequency of standard types. In translocation heterozygotes of *Pisum* giving 50 % of sterility (the random expectation being 66,7 %) it may be assumed that $a = {}^1/_2$ and $b = c = {}^1/_4$, or more generally $a = 2b$. This gives $p = \frac{x}{4 - x}$ which, if x is small as compared with 4, could be written as $p = \frac{x}{4}$.

If the chiasma frequency of the interstitial segment in this case is just as high as in standard lines, the p-value would nevertheless be reduced by one half. This could perhaps, as mentioned earlier, partly explain the reduction of St—26 %—B to St—12,8 %—B. In the translocation heterozygote between structural type 1 (the standard type) and

structural type 6 (cf. SANSOME, 1937) a chiasma is often formed between the centromere and the point of interchange in one pair of chromosomes in the association (SUTTON, 1935). Incidentally it might be mentioned that a regular zigzag orientation ($a = 1$) would give $p = 0$, whereas p would rise to 1 if B_1 was the only orientation occurring.

In these calculations the simultaneous occurrence of two chiasmata in the interstitial segment has not been taken into consideration. Neither has proper allowance been made for the possibility that interstitial chiasmata may act upon the orientation of the ring of four. Most of the computations presented in this paper are based on assumptions that may only very approximately correspond to the actual conditions in the plants investigated. For the purpose of comparisons between various progenies and for studies of the gene sequence in relation to the point of interchange they may, however, be of some value.

SUMMARY.

Detection and estimation of linkage in the F_2 progeny of a selfed translocation heterozygote is demonstrated and a comparison is made with the corresponding data from a backcross progeny.

In the translocation studied, which seems to be that of Extra Rapid, the point of interchange is situated to the left of the chromosome section St—B. Within this section the crossing-over values are rather reduced in the translocation heterozygote when compared with the standard structural type. The causes of this reduction are discussed.

LITERATURE CITED.

1. HÅKANSSON, A. 1931. Über Chromosomenverkettung in *Pisum*. — Hereditas XV: 17—61.
2. — 1934. Chromosomenbindungen in einigen Kreuzungen zwischen halbsterilen Erbsen. — Hereditas XIX: 341—358.
3. LAMPRECHT, H. 1942 a. Die Koppelungsgruppe *Gp—Cp—Fs—Ast* von *Pisum*. — Hereditas XXVIII: 143—156.
4. — 1942 b. Genstudien an *Pisum sativum*. V. Multiple Allele für Punktierung der Testa: Fs_{ex}—*Fs*—*fs*. — Hereditas XXVIII: 157—164.
5. — 1946. Die Koppelungsgruppe *Uni—M—Mp—F—St—B—Gl* von *Pisum*. — Agri Hortique Genetica, Bd. IV: 15—42. (Reports from the Plant-Breeding Institution Weibullsholm, Landskrona, Sweden.)
6. MATHER, K. 1938. The measurement of linkage in heredity. — Methuen's monographs on biological subjects. Methuen & Co. Ltd., London.

7. MATHER, K. 1946. Statistical analysis in biology. Second edition. — Methuen & Co. Ltd., London.
8. NILSSON, E. 1936. Erblichkeitsversuche mit *Pisum*. IX. — Hereditas XXI: 167—184.
9. PELLEW, C. 1940. Genetical studies on the first reciprocal translocation found in *Pisum sativum*. — Journ. of Genetics XXXIX: 363—390.
10. SANSOME, E. 1932. Segmental interchange in *Pisum sativum*. — Cytologia, Vol. 3: 200—219.
11. — 1937. Segmental interchange lines in *Pisum sativum*. — Nature, Vol. 139: 113.
12. SUTTON, E. 1935. Half-disjunction in an association of four chromosomes in *Pisum sativum*. — Annals of Bot. XLIX: 689—698.

CONTENTS.

Introduction 280
Survey of the investigations 280
Detection of linkage 282
Estimation of linkage 282
Interpretation and discussion of the results 285
Summary 287
Literature cited 287

A CONTRIBUTION TO THE GENETICS OF CONGENITAL CLUBFOOT

BY *J. A. BÖÖK*

DEPT. OF MEDICAL GENETICS, INSTITUTE OF GENETICS, LUND

CONGENITAL clubfoot is the commonest serious deformity of the foot, and it has been the subject of many investigations; surveys are given by DEBRUNNER (1936), LANGE (1935), KREUZ (1939), OSTERTAG (1939) *et alii*. The frequency of this malformation has been estimated at 1/1200 new born (cf. BAUER and BODE, 1940) or in Swedish material at 1/1000 (NILSONNE, 1927). In about 50 per cent of cases both feet are affected.

The old conception was that congenital clubfoot was caused by forced positions *in utero*. As possible causative agents were cited: a reduced amount of amnion fluid, strangulations by the umbilical cord, amnion strings, etc. Though it can not be denied that very seldom congenital clubfoot may be caused purely mechanically, recent clinical research combined with genetical analysis has shown, beyond a reasonable doubt, that most cases should be referred to the presence of genes resulting in the defect (cf. IDELBERGER, 1939).

TWIN RESEARCH.

In 1939 IDELBERGER published a very extensive twin study. Starting with 11459 cases he reached a series of 35 monozygotic and 133 dizygotic twin pairs. He examined the whole material personally. The main results are compiled in Tables 1 and 2. The significant difference between monozygotic and dizygotic pairs shows clearly the importance of the genotype, though the frequency of manifestation is only 32,5 per cent (calculated according to WEINBERG's propositus method).

Nothing extraordinary could be found concerning pregnancy or parturition. The frequency of twins in the whole material was not significantly raised as compared with a normal population. This all strongly suggests that the conception given above is correct.

It should also be observed that the frequency of manifestation is twice as high in men. This difference, however, is not statistically significant but is confirmed by the long known fact that the sex ratio

TABLE 1. *Twins with congenital clubfoot. Data from* IDELBERGER (*1939*).

Twins	Concordance	Discordance	Total	Concordance per cent
Identical	13	27	40	32,5
Fraternal	4	130	134	2,9

$\chi^2_{corr} = 66{,}4$; $P < 0{,}001$.

TABLE 2. *Identical twins with congenital clubfoot. Data from* IDELBERGER (*1939*).

Identical twins	Concordance	Discordance	Total	Concordance per cent
Male	6	16	22	27
Female	2	11	13	15

in every large clubfoot material is two men: one woman (25000 cases compiled by MAU; see BAUER and BODE, 1940).

FAMILY RESEARCH.

With a manifestation frequency of 32,5 per cent we could *a priori* not expect simple answers in studying pedigrees or series of sibships. It should also be stressed that this figure has a comparably great mean error ($\pm$ 7,3 %).

More extensive family studies have been reported by FETSCHER (1922), MÜLLER (1926), ISIGKEIT (1927) and ASSUM (1936). They have all personally examined only a small part of their families. Most of the material has been collected by means of correspondence with members of the families, authorities and hospitals. MÜLLER declares that he has personally visited only those families from which he heard that there were further cases of clubfoot. This makes a false selection and his material cannot be used here.

FETSCHER's material includes 184 *propositi*. In three cases the father was also affected, in the rest both parents were unaffected. Of 181 *propositi* with unaffected parents 147 had together 634 sibs, and among these 17 were affected with congenital clubfoot. This makes an empirical risk of 2,7 per cent. FETSCHER writes 3,06 per cent, owing to a minor error in his calculation. The frequency of marriages between 1st cousins among the parents is 2,2 $\pm$ 1,1 per cent, a value which is not significantly different from the mean of the population at that time in Germany.

In the material of ISIGKEIT, apparently, 400 *propositi* (including secondary propositi) had 824 sibs and from these 25 were affected, the parents being unaffected in all these cases. This makes a frequency of 3,03 per cent. Three cousin marriages are communicated. It is, however, not possible to calculate the frequency out of the data given. The primary material is not reported.

ASSUM communicates 50 *propositi*, 40 of which had together 117 sibs. Of these 4 were affected, i. e. 3,4 per cent. In his secondary cases two abortions with clubfoot are included. Only one of these is included in his calculation. The meaning of this is not understandable. For a genetical investigation both must be taken into account. In his material we find one marriage between 1st cousins.

TABLE 3. *Marriages between unaffected parents with at least one child with congenital clubfoot. Collection of data.*

Author	No. of propositi	Sibs total	Affected sibs	Clubfoot per cent	No. of 1st cousin marriages	Frequency of 1st cousin marriages
FETSCHER (1922)	184	634	17	2,7	4	$2{,}2 \pm 1{,}1$ %
ISIGKEIT (1927)	400	824	25	3,0	3	
ASSUM (1936)	50	117	4	3,4	1	
Total:	624	1525	46	$2{,}9 \pm 0{,}4$	8	

The data of these three authors are compiled in Table 3. The correspondence is very good. We are further in a position to be able to control this material by comparing it with the data of IDELBERGER. In doing this we will see that the frequency of affected dizygotic twin partners is just 2,9 per cent, thus confirming the results of the above mentioned family studies. IDELBERGER himself has taken a value of 5 per cent from the publications of FETSCHER and ISIGKEIT, evidently without observing minor calculation errors.

We may thus be justified in considering the empirical risk figure as 3 per cent, or in other words: *if the parents are unaffected and already have one child with congenital clubfoot the mean risk of following children being affected with the same malformation is 3 per cent.* Under these circumstances the risk is about 36 times greater than it should have been by mere chance.

In FETSCHER's material the frequency of 1st cousin marriages is $2{,}2 \pm 1{,}1$ per cent. The frequency in the population is estimated at

0,5 per cent (LENZ, 1936, p. 619). The difference is not significant. If we take IDELBERGER's conception of a simple recessive with a low penetrance the frequency of the *RR*-genotypes should be $\frac{1}{1200 \times 0{,}325}$, i. e. 0,003. The frequency of 1st cousin marriages may then be calculated according to KOLLER (1940, p. 297) as

$$P = \frac{v\ (1 + 15\ q)}{16\ q + v\ (1 - q)}$$

where P is the expected frequency and v the frequency of 1st cousin marriages in the population and q the frequency of the *RR*-genotypes. The formula is valid of course only on the assumption of free pairing. P is calculated to 11 per cent. This finding does not correspond with the conception of one simple recessive gene, spread all over the population.

The conception of IDELBERGER is further that we are dealing with a gene having a strong sex limitation. There is no indication of sex-linked inheritance. A new examination of the available data by the present author gives no indication that both types of inheritance may be present.

THE PRESENT PROBLEM.

In medical genetics it is practice to start with a clinical syndrome. Those patients in which an apparently exogenous cause of the disease is found are excluded from the beginning. This is justified. Later, however, the rest showing a more or less homogeneous clinical picture are often treated as if representing also a genotypical entity. This conception, of course, is not true without further genetical analysis.

The calculation of an empirical risk figure is no doubt of a practical value, and in many cases we get only just to this point. If we realize that our *propositi* material in spite of all precautions still may be heterogeneous genotypically it is not to be wondered at that there is often no correspondence to the Mendelian figures, and this applies even if it should happen that we are dealing with one simple pair of genes, i. e. if the mode of inheritance is simple recessive or dominant. Entirely different genes (at different loci) or different alleles may be operating in different families, genes or alleles with different frequency of phenotypical manifestation. Considering only the genes connected with the disease in question, there is a great risk that the material may be genotypically heterogeneous. A material, however large, therefore must

have its complement in an analysis of *inter* and *intra* familial variability. In large populations where we in reality have to do with several minor populations or isolates the geographical variability of the disease must also be taken into account.

If at first we accept IDELBERGER's hypothesis that congenital clubfoot is a simple recessive, then are we dealing with just *one* gene with a frequency of about 5,5 per cent in the population ($\pm\sqrt{0{,}003}$) and a homozygotic manifestation frequency of about 32,5 per cent?

In spite of the very extensive twin material we must not forget that the figure arrived at is connected with a comparably great mean error. Statistically this means that the actual manifestation frequency is something between 10,6 and 54,4 per cent. The frequency of affected sibs in the present material lies between 1,7 and 4,1 per cent. This is based on the conventional consideration of practically the whole variability lying between $\pm$ three times the mean error. If we calculate on the basis of these figures the probable frequency of *RR*-genotypes among the sibs of patients with congenital clubfoot (parents unaffected) the highest value is 38,7 per cent $\left(\frac{4{,}1}{0{,}106}\right)$ and the lowest 3,1 per cent $\left(\frac{1{,}7}{0{,}544}\right)$. So there is still no proof against the conception of a simple recessive inheritance. Whether the congenital clubfoot is a genotypical entity is, however, still obscure.

Hypotheses concerning dimeric or polymeric inheritance should be rejected until a monomeric inheritance is disproved.

We must assume that genotypically controlled defects and diseases arise through a mutation process, i. e. in most cases a gene mutation. The occurrence of mutations in human populations has been shown with a very high degree of probability, for instance for chondrodystrophy (MÖRCH, 1941). The mutation frequency was first calculated by HALDANE (1935) for *haemophilia* and estimated at 1/50.000 to 1/100.000 X-chromosome. This is in a very good agreement with the results of the experimental genetics.

Now it is reasonable to assume that also congenital clubfoot arises through gene mutations. If this process is still going on is not possible to settle without knowing the reproduction capacity of the affected individuals. No data of this kind are yet available but it is probable that the reproduction is slower than for healthy individuals. The relatively constant proportion of about 1/1200 new born would then allow a calculation.

Mutations occurring in different parts of a population do not need

to be identical. As for *haemophilia* we know two types, one severe and one slight, which are connected with different alleles. This means that minor differences in the genes also are connected with differences of the manifestation. In a way this is a truism but a truism that has not been enough realized.

If, for instance, we think of three recessive alleles, R_1, R_2 and R_3, located in a somatic chromosome, it is very probable that the six possible combinations represent variations in the character. The manifestation frequency may be different in different gene constellations. Conditions of this kind could not be unveiled if in the usual way a great number of sibships are examined and the starting point is a more or less obscure syndrome. Neither will twin examinations avail anything here. The only satisfactory method of analysis is by family and pedigree studies.

There is, however, another complement in the analysis of the genotypical entity of a clinical syndrome. If this is connected with a simple recessive gene pair which is identical all over the population, then the risk figure for the sibs of the affected individuals (parents unaffected) must be the same if the parents are related or not. If this is not the case it is reasonable to doubt the genotypical entity.

In ASSUM's (1936) and FETSCHER's (1922) material there are three sibships where the parents are 1st cousins. These families have been obtained without selection. We shall add a fourth family examined by the present author. This family, called the X-järvi family, was obtained in another investigation concerning the registration and examination of all marriages between 1st cousins in a certain area in North Sweden (three parishes with 9190 inhabitants). Congenital clubfoot occurred only in this family of my material. The frequency of marriages between relatives is comparably high in this area. The figure for 1st cousin marriages is $2{,}21 \pm 0{,}41$ per cent.

Table 4 shows the frequency of congenital clubfoot among the sibs in the two constellations if the parents are 1st cousins or not related. The difference is significant in spite of the cousin material being very small.

In principle there seems to be two reasonable explanations of this fact. In my opinion it is most probable, according to the reasoning given above, that the genotypical background of congenital clubfoot is heterogeneous. If we have in a population different recessive genes for congenital clubfoot with a varying penetrance and those having the highest penetrance are very rare, this is what we should expect to find.

TABLE 4. *Frequency of congenital clubfoot.*

Parents (unaffected)	Unaffected sibs	Affected sibs	Sibs total	Clubfoot per cent
1st cousins	11	4	15	26,6
Unrelated	1514	44	1558	2,9

$P = 0{,}003$.

The very rare recessive will come out in homozygous form mainly in marriages between relatives. The conception of different alleles will be a special instance of this explanation only.

The second possible explanation is that other rare recessive genes are able to raise the manifestation frequency. Let R_1 be a recessive gene with weak penetrance and its frequency 0,055 (compare above where the frequency of clubfoot *genotypes* is estimated at 0,003). $\overline{D_1R_1}$ (= the frequency of D_1R_1-genotypes) is then 0,1 and the chance for $D_1R_1 \times D_1R_1$ marriages 0,01 if we exclude the small possibility of assortative mating. Of these marriages most R_1R_1 genotypes would not manifest the defect. Let further R_2 be another recessive gene in another chromosome which raises the penetrance, i. e. $R_1R_1R_2R_2$ genotypes will manifest the defect in a higher frequency. Suppose $\overline{R_2}$ is 0,01 and $\overline{D_2R_2}$ 0,02. The chance of being $D_1R_1D_2R_2$ then is 0,002 and the chance for a mating between two such double heterozygotes would approximatively be 0,000004. The meaning of this is only that the R_2 gene would be so rare that R_2R_2 genotypes as a complement to R_1R_1 should be realized mostly after inbreeding. Phenotypically this means a higher manifestation frequency if the parents are related.

Which one of these explanations that holds good is not possible to decide at the moment. Anyway the conclusion is that the entity of the genotypical background of congenital clubfoot is highly dubious.

Practically this means that the empirical risk figures which we get through the *propositus* method of WEINBERG should be handled with some caution. Not only the parent—sibship constellation but also the pedigree should be realized.

THE X-JÄRVI FAMILY.

This is a family of lumbermen in North Sweden, on the Finnish border. The family was examined by the present author in January, 1947. The research work in the Church Register as well as the field

work has been faciliated by the interest and kind assistance of the Rev. O. HAAPANIEMI of Pajala.

The family is included in an investigation of all marriages between first cousins of three parishes (9190 inhabitants; cf. BÖÖK, 1948). Thirty-two such marriages have been found and examined and up to now a control material of 35 marriages between unrelated persons, taken haphazardly, has been added. Cases of congenital clubfoot have been found in no other family.

The figures below refer to the pedigree (Fig. 1).

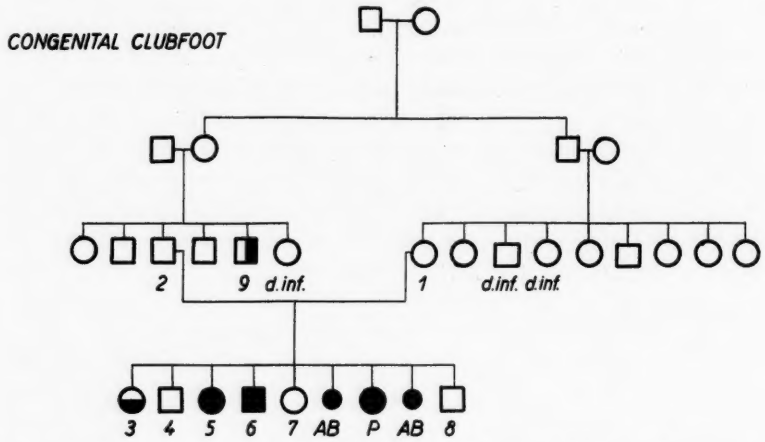


Fig. 1. Pedigree of the X-järvi family. ■, ●, bilateral clubfeet. *AB*, abortion. *D. inf.*, died in infancy. Further discussion in the text.

P. Named propositus. — ♀ Aina E. M. Born 1918 1/3. The note in the Church Register is congenital clubfeet, diagnosis by the midwife. Normal parturition. She was first examined by a physician at school when she was seven years old. No treatment was started. In 1936 she visited the Orthopedic Clinic at Härnösand (No. 6962/36): severe clubfeet. The forefeet turned in maximum *varus* position, adduced and torqueted so that the patient stands on the *dorsum pedis*. The heels in a maximum *equinus* position. (Compare Fig. 2.) Prominent subjective disorders. However, she did not want any treatment, nor orthopedic shoes. Later on her condition was still impaired and she could hardly walk owing to *clavi*. Her working ability was impaired too, and in Jan. 1939 she visited the district physician for a State Pension. A complete physical and mental examination was performed and her working capacity considered to be reduced to 1/3 normal. The patient was recommended to visit the Orthopedic Clinic for an operation. This was performed later the same year as an attempt to improve her condition. Operation report: A wedge resection of the right *tarsus* and extirpation of *talus* and *naviculare*.

After *achillo-tenotomia* the position of the foot was relatively good. Plaster. Later the patient got necroses and infection in the foot and it was necessary to amputate both legs. — *Catamnesis.* Physical and mental examination no remark. Locally: both legs amputated 16 cms below the knee joints, good mobility here. Mobility in the hip joints no remark. She has now protheses on which she walks relatively good.

No. 1. ♀ Maria M. M. Born 1881 $^{2}/_{8}$. Mother of the *propositus* and first cousin of No. 2. Always healthy. Seven normal parturitions and two early spontaneous arbortions. Never been ill during the pregnancies. She has normal feet. A complete physical examination is rejected. My impression is a medium degree mental inferiority. This impression of her mental state was confirmed later by the

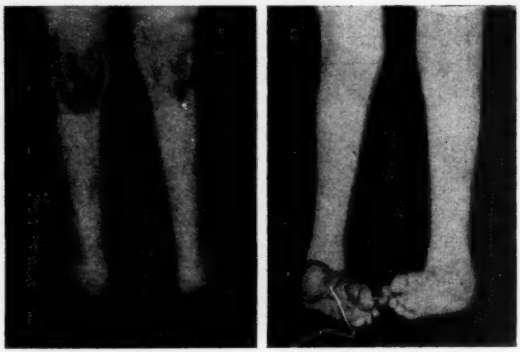


Fig. 2. Typical bilateral clubfeet of the *propositus.*

opinions of the local authorities and the district nurse. Probable diagnosis: *Debilitas mentis.*

No. 2. ♂ Isak A. M. Born 1875 $^{17}/_{7}$, died 1944 $^{15}/_{2}$ of cardiac failure and cerebral haemorrhage. Father of the *propositus.* In Oct. 1938 he went to the district physician for a State Pension, and was submitted to a thorough examination. Since 1928 had been able to do only light work. Easily short of breath when walking. Subjective cardiac disorders with pain in the chest and anxiety. Physically: General constitution no remark. *Cor*: Very feeble heart sounds. Deficient pulse. Blood pressure 250/130 mm Hg. X-ray: emphysema of the lungs and enlargement of the heart. Neurologically no remark. Mentally prominent neurastenic characteristics. Nothing has been noted about the feet, but as a very scrupulous examination is required for this kind of certificate any malformation of the feet can be ruled out.

No. 3. ♀ Maria M. M. Born 1907 $^{18}/_{3}$. She has left the village and it was not possible to arrange an examination. According to her school teacher, she had some kind of a malformation of one foot. The district nurse reports that there is no clubfoot but probably a congenital *pes planovalgus.* Pronounced mental deficiency. She has 6 children, all physically normal. Probable diagnosis: *Debilitas mentis.*

No. 4. ♂ Elis A. M. Born 1908 $^{30}/_{12}$. Since 1927 periodically gastric ulcer disorders. At the examination no subjective disorders. Physically and mentally no remark. Normal feet.

No. 5. ♀ Fanny E. M. Born 1911 $^{9}/_{2}$, died a few hours after parturition. She had typical clubfeet. This diagnosis made by the midwife was found in the Church Register too.

No. 6. ♂ Oskar A. M. Born 1913 $^{30}/_{11}$. Diagnosis by the midwife in the Church Register: congenital bilateral clubfeet. Examined at the Orthopedic Clinic at Härnösand 1932. The feet are shown in Fig. 3. The clinical picture is almost identical with the *propositus*. Amputation was suggested but rejected. Instead he got orthopedic shoes. *Status*: Severe untreated typical clubfeet. Walks on the *dorsum pedis*. Physically and mentally no remark. Doing well as a shoemaker.

No. 7. ♀ Aili E. M. Born 1915 $^{11}/_{12}$. Healthy. Physically and mentally no remark. Normal feet.

Fig. 3. The feet of No. 6 in the pedigree.

No. 8. ♂ Karl E. M. Born 1921 $^{28}/_{1}$. Healthy. Physically and mentally no remark. Normal feet.

No. 9. ♂ Axel V. M. Uncle of the *propositus*. Born 1884 $^{3}/_{4}$, died 1892 $^{24}/_{7}$. According to a note in the Church Register, he had »a malformation of one foot». No other information is available.

Summary of family history. — Marriage between 1st cousins with normal feet. Mental deficiency of the mother. From seven children born alive three have congenital bilateral clubfeet. There have been no disorders during the pregnancies and the parturitions have been normal.

SUMMARY.

(1) The literature of the etiology and genetics of congenital clubfoot is reviewed.

(2) The frequency of individuals with congenital clubfoot among

the sibs of clubfoot *propositi* (parents unaffected) is significantly raised when the parents are first cousins (material from investigations by FETSCHER, ISIGKEIT, ASSUM and the present author).

(3) The above finding is discussed and the conclusion is that the genotypical background of congenital clubfoot is probably heterogeneous.

(4) Practically we must realize that the mean empirical risk figure (3 per cent) may be raised if the parents are related. Especially if the parents are first cousins and already have got one child with congenital clubfoot the prevention of further children by sterilization or *abortus provocatus* should be allowed if desired.

LITERATURE CITED.

1. ASSUM, H. W. 1936. Untersuchungen über die Erblichkeit des angeborenen Klumpfussleidens. — Z. Orthop. Bd. 65, pp. 1—42.
2. BAUER, K. H. and BODE, W. 1940. Erbpathologie der Stützgewebe beim Menschen. — Hdb. Erbbiol. d. Mensch. Bd. 3, pp. 231—269.
3. BÖÖK, J. A. 1948. The frequency of cousin marriages in three North Swedish parishes. — Hereditas XXXIV: 252—255.
4. DEBRUNNER, H. 1936. Der angeborene Klumpfuss. — Deutsche Orthopädie, Bd. 10. 131 pp.
5. FETSCHER, R. 1922. Über die Erblichkeit des angeborenen Klumpfusses. — Arch. Rass.- u. Ges.-Biol. Bd. 14, pp. 39—52 and pp. 146—158.
6. HALDANE, J. B. S. 1935. The rate of spontaneous mutation of a human gene. — Journ. of Gen. Vol. 26, pp. 317—326.
7. IDELBERGER, K. 1939. Die Zwillingspathologie des angeborenen Klumpfusses. — Z. Orthop. Bd. 69, Beil. — H. 1—3, 95 pp.
8. ISIGKEIT, E. 1927. Untersuchungen über die Heredität orthopädischer Leiden. — Arch. Orth. u. Unfall-Chir. Bd. 15, pp. 535—556.
9. KOLLER, S. 1940. Methodik der menschlichen Erbforschung. — Hdb. Erbbiol. d. Mensch. Bd. 2, pp. 249—309.
10. KREUZ, L. 1939. Das derzeitige Wissen vom Wesen des angeborenen Klumpfusses und der angeborenen Hüftgelenkverrenkung. — Z. Orthop. Bd. 69, Beil. — H. 1—3, pp. 199—220.
11. LANGE, M. 1935. Erbbiologie der angeborenen Körperfehler. — Z. Orthop. Bd. 63, Beil. — H. 1—2, 143 pp.
12. LENZ, F. 1936. Die krankhaften Erbanlagen. — BAUR—FISCHER—LENZ: Menschl. Erblehre u. Rassenhygiene, Bd. 1, pp. 323—586.
13. MÜLLER, W. M. 1926. Zur Ätiologie des angeborenen Klumpfusses unter besonderer Berücksichtigung seiner Vererbung. — Arch. J. Klausst. Bd. 2, pp. 1—37.
14. MÖRCH, E. T. 1941. Chondrodystrophic dwarfs in Denmark. — Munksgaard, Copenhagen.

15. NILSONNE, H. 1927. Eine statistische Studie über den kongenitalen Klumpfuss. — Z. orth. Chir. Bd. 48, pp. 219—228.

16. OSTERTAG, M. 1939. Das derzeitige Wissen vom Wesen des angeborenen Klumpfusses und der angeborenen Hüftgelenkverrenkung. — Z. Orthop. Bd. 69, Beil. — H. 1—3, pp. 232—242.

CONTENTS.

Twin research 289
Family research 290
The present problem 292
The X-järvi family 295
Summary 298
Literature cited 299

A GENETICAL ANALYSIS OF SOME SPELTOID STRAINS

BY *Å. ÅKERMAN* AND *J. MAC KEY*
WHEAT AND OAT DEPARTMENT OF THE SWEDISH SEED ASSOCIATION, SVALÖF

IN his paper of 1917 NILSSON-EHLE drew attention to an off-type isolated from *Triticum vulgare*. The deviating plants were characterized by short outer glumes, almost square across the top, strongly keeled and with green stripes running down towards the base, thus resembling *Triticum Spelta*. He, therefore, called them speltoids. He (1917, 1920, 1921) proved them to have arisen through mutation and grouped them according to the progeny ratio of the selfed speltoid heterozygote into three genetic types. The A type is characterized by a segregation approaching 1 : 2 : 1 of normals, het speltoids and hom speltoids. B het speltoids give about four or five times as many heterozygous as normal segregates and only a very few hom speltoids. In addition subcompactoids, i. e. short dense-eared plants resembling *Triticum compactum*, occur as rare but fairly regular segregates. C het speltoids usually give as many normal as heterozygous offspring, and again only a few weak and more or less sterile hom speltoids. Fresh reviews of the hypotheses on the origin and the relationship of the speltoid and compactoid off-types are given by HUSKINS (1946) and by SÁNCHEZ-MONGE and MAC KEY (1948).

An extensive genetical analysis of different speltoid series (NILSSON-EHLE, ÅKERMAN, LINDHARD, KAJANUS *et al.*) rather soon showed the complexity of these mutations. The segregation behaviour of the series could not exclusively be explained as true gene mutations; deficiencies and other chromosome aberrations must be assumed. The speltoid and compactoid investigations also turned more and more over to cytology. As material for these cytological studies speltoid strains originating from Svalöf have been very often used (WINGE, HUSKINS, HÅKANSSON, MÜNTZING, UCHIKAWA, SÁNCHEZ-MONGE and MAC KEY). The genetical analysis of the spring wheat strains supplied by the senior writer was not immediately published, as it was considered impossible to explain the problems concerned without a thorough cytological examination of the material. Owing to intervening work the report of the genetical analysis has, however, been successively postponed. The origin of the

three types of speltoid wheats seems now quite definitely explained by the work carried out during the two last decades. Therefore, the present much delayed paper, in which these ratio tests are now given, must be looked upon more as a complementary background to the cytological studies performed than as a direct contribution to the explanation of the speltoid and compactoid mutations.

Owing to the accounting nature of the present publication of the ratio tests it has not been considered reasonable to present all the gathered data in detail. The tables showing the different segregation ratios, therefore, only give the bulk progeny figures per year. No difference in the progeny ratio has been found between, e. g., het speltoids derived from het speltoids or from subcompactoids or subcompactoids derived from het speltoids or from subcompactoids. When bulking the figures no consideration has therefore been paid in the tables to the different origin. The collective field numbers given in the tables refer to the year of the progeny test. The tested plants are always selected in the plots of the preceding year with only one exception. So the tests of both the years 1927 and 1928 comprise plants of the year 1926. For a detailed study of the progeny tests performed the writers refer the readers to the records of the Wheat and Oat Department, Swedish Seed Association, Svalöf, Sweden, where a collocation of all the figures is available.

The heterogeneity between years of the different progeny series has been estimated. As the range of years and the number of progeny plants per year are varying, the P-values for the different series are not quite comparable. Rare segregates (less than 5 expected plants per year) have been excluded from the χ^2-analysis. Years with a very small number of plants have been combined with the subsequent year.

THE SPELTOID STRAIN FROM BROWN SCHLANSTEDT × 0715.

In a preliminary yield trial of the year 1917 involving selection from the spring wheat cross Brown Schlanstedt × 0715 (a purebred line of the Norwegian variety Börsum), speltoid plants were observed in two of the lines. In both cases the original mutation must have happened at least in the year 1915. Several plants, both het speltoids and hom speltoids, were observed evenly distributed to the three replications of the lines. Both speltoid families showed an A type segregation with a relatively low frequency of het speltoids. In Table 1 the result of the

continuous segregation test based on one original het speltoid is given. Normal and het speltoid plants are beardless, hom speltoids are bearded. As seen from the table, the strain is of a very constant type. No secondary mutations have been observed. The segregation ratio seems very little influenced by environmental factors.

TABLE 1. *The progeny ratio of selfed A het speltoids (original mutation arisen in the spring wheat cross of Brown Schlanstedt* × *0715).*

Field number	No. of plant progenies tested	No. of progeny plants				No. of plants harvested in % of kernels sown
		normals	A het speltoids	A hom speltoids	total	
1918—397	1	17	10	1	28	54
1919—486	6	78	107	18	203	57
1920—393	11	274	368	70	712	55
1921—192	6	112	152	26	290	51
1922—423	5	155	189	29	373	43
1924—443	13	284	375	92	751	75
1926—446, 447	31	946	1156	242	2344	74
1927—444	10	332	383	86	801	87
1928—418, 419	39	1267	1494	347	3108	74
1929—417	10	322	416	90	828	67
1930—424	15	366	479	83	928	40
1932—420	10	156	221	44	421	50
1935—451	3	22	38	9	69	15
1937—950	3	134	159	34	327	78
1939—951	3	125	131	30	286	88
1941—951	3	98	124	30	252	80
1945—951	3	45	62	13	120	43
1947—962	3	177	223	57	457	73
Total	175	4910	6087	1301	12298	67
Ratio		1 :	1,24 :	0,26		

Index of heterogeneity, $\chi^2 = 25{,}8807$; d. f. $= 32$; $0{,}80 > P > 0{,}70$

The strain has been cytologically examined by WINGE (1924) and HUSKINS (1927, 1928). A chromosome count was also carried out last summer. All investigations have proved both the het speltoid and the hom speltoid to have 42 chromosomes. The chromosome behaviour was normal but both WINGE and HUSKINS found occasional irregularities at the meiosis. The most significant of these appeared to be the formation of $19_{II} + 1_{III} + 1_{I}$ in the het speltoid and $19_{II} + 1_{IV}$ in the hom speltoid. Together with the same experiences with an A strain from LINDHARD

these observations led WINGE to his hypothesis of the B and C chromosome substitution, an explanation of the arising of speltoids to which HUSKINS (l. c.) fully agreed. Later on, however, NISHIYAMA (1931), working with the related A type of fatuoids, and UCHIKAWA (1941) established that the meiotic irregularities of the A type bore no relation to the plant phenotype and therefore gave no basis for the substitution hypothesis. In a recent paper HUSKINS (1946) also considers the chromosome substitution scheme as a worn-out theory.

The deviation from 1 : 2 : 1 in the progeny ratio of A het speltoids was explained already by NILSSON-EHLE (1920) as due to failure of speltoid-bearing pollen to function at the fertilization. This was confirmed by ÅKERMAN (1923) on the basis of the reciprocal cross normal × het speltoid. The same proof can be given by the reciprocal cross het speltoid × hom speltoid. In the Brown Schlanstedt × 0715 strain the cross het speltoid × hom speltoid gave quite equal numbers (31 : 34) while the reciprocal cross gave excess of het speltoids (39 : 10). Supposing the speltoid-bearing gametes to be just as effective as the normal ones on the female side but four times weaker on the male side, the segregation 1 : 1,25 : 0,25 should be expected. This is quite in agreement with the ratio given in Table 1.

The different competitive ability of the two pollen types can also be illustrated by the progeny of free-flowering normal and hom speltoid plants. Here a certain though rather low frequency of natural incrossing always occurs. Out of 2957 progeny plants of normals only 3 (0,1 %) het speltoids were observed (here a new mutation may also be a possible explanation) but out of 2348 descendants from hom speltoids 31 (1,3 %) were observed to be het speltoids, thus showing the higher competitive ability of the normal pollen.

THE SPELTOID STRAIN FROM 0715.

From an observation plot of the above-mentioned line 0715 of Börsum two het speltoid plants were isolated in 1917. The progeny test showed them to be of the B type, one giving 7 normals and 42 het speltoids in the offspring, the other only 15 het speltoids. In Table 2 the continuous segregation test based on the first-mentioned original het speltoid is given. Only the hom speltoids are bearded. The index of heterogeneity given in the table is based only on normal and het speltoid offspring owing to the very low frequency of the other segregates.

As seen from the table, the speltoid strain out of 0715 behaves as a

TABLE 2. *The progeny ratio of selfed B het speltoids (original mutation arisen in the spring wheat strain of 0715).*

Field number	No. of plant progenies tested	No. of progeny plants: normals	B het speltoids	B hom speltoids	subcompactoids	chimaeras	total	No. of plants harvested in % of kernels sown
1918—397	1	7	42	—	—	—	49	34
1919—498	21	72	225	—	1	1	299	50
1920—400	44	574	2180	17	5	7	2783	33
1921—188—190	10	55	266	—	—	—	321	53
1922—421	12	167	621	7	1	1	797	47
1923—431	34	193	810	—	9	1	1013	35
1924—440—442	120	1337	5099	15	1	11	6463	45
1926—440—445	65	937	4397	80	2	7	5423	71
1927—438—443	38	672	2948	9	7	4	3640	75
1928—420, 425, 428	17	208	878	13	—	5	1104	80
1929—418, 426, 427	23	246	1296	46	7	2	1597	64
1930—424, 425	25	407	1635	41	15	1	2099	33
1932—421, 422	17	303	1330	53	1	4	1691	38
1935—452, 454	7	80	403	4	5	1	493	55
1937—951	6	85	349	6	2	—	442	56
1939—952	6	114	480	15	4	2	615	76
1941—952	6	77	363	4	—	—	444	56
1945—952	7	42	184	—	—	—	226	22
1947—963	5	79	391	4	3	—	477	67
Total	464	5655	23897	314	63	47	29976	52
Ratio		1 :	4,23 :	0,06 :	0,01 :	0,01		

Index of heterogeneity, $\chi^2 = 50{,}2008$; d. f. $= 18$; $P < 0{,}001$

typical series B except in the very high frequency of chimaeras observed. All of these were mosaics of normal and speltlike tissues. The origin of this type of chimaera may be explained either as a somatic elimination of one C-chromosome in originally normal plants or as a somatic non-disjunction of the C-halves in originally het speltoid plants. The latter explanation seems to be the more probable one, for otherwise chimaeras must be expected to be just as common also in the progeny of normal plants. The problems concerning the speltoid chimaeras have earlier been discussed by ÅKERMAN (1920, 1927).

The genetical analysis of the speltoid strain of 0715 is quite in harmony with the cytological examination made by HUSKINS (1928), HÅKANSSON (1930, 1932) and recently by SÁNCHEZ-MONGE and MAC KEY (1948; equal to their series I). In all investigations the het speltoid was observed to have 41 chromosomes, at metaphase I quite regularly forming a $20_{II} + 1_{I}$ configuration. The few cases of $19_{II} + 3_{I}$ or $19_{II} + 1_{III}$ observed do not constitute more irregularities than usual in common *vulgare* strains. The excess of het speltoids, characteristic of the segregation type of the B-series, has in the case of the 0715 strain as well been shown by the above-mentioned workers to be caused by the high tendency for the C-univalent to be left out of the daughter nuclei at meiosis. From the cross het speltoid $\times$ normal in another B-series UCHIKAWA (1941) has found that egg-cells with 20 chromosomes are four times as frequent as those with 21 chromosomes. This is in agreement with the result from the same intratypic cross performed with the present strain. Here, 24 normals and 90 het speltoids were found in F_1. In the reciprocal cross UCHIKAWA only got 18 F_1 plants, indicating the ratio of the two phenotypes being approximately 1 : 5. From these results he calculated the zygotic ratio to be 1 normal : 4,2 het speltoid : 0,8 hom speltoid. The reciprocal cross performed for the present strain gave a much more pronounced excess of normals (200 : 10). A calculation on the basis of the present figures will thus give a zygotic ratio of 1 : 4,05 : 0,2. The validity of this re-examination of the functioning ability of the speltoid-bearing pollen of series B will be discussed in General Conclusions.

Besides chimaeras, subcompactoids have been observed as fairly regular but rare secondary mutations out of het speltoids (here in 0,21 % of the offspring). HUSKINS (l. c.) found in a preliminary count 42 chromosomes in two subcompactoid plants examined. Except one quite anomalous case with $20_{II} + 1_{I} + 1$ fragment HÅKANSSON (l. c.) found two different karyotypes of the subcompactoids. The more common

type, the normal subcompactoid, was observed to have the chromosome complement 40 + no + co, the no-chromosome being a C-chromosome and the co-chromosome later on proving to be an iso-chromosome (in HUSKINS's paper of 1946 called Cil). In a recent re-examination of the 0715 strain performed by SÁNCHEZ-MONGE and MAC KEY (l. c.) the same karyotype of the subcompactoids has been observed. The origin of these subcompactoids from B het speltoids has been discussed in their paper.

The second subcompactoid karyotype of HÅKANSSON, only once (1923; his plant 1930—425—32 B) observed in the present series, had 43 chromosomes, was called a trisomic subcompactoid and was supposed to have a chromosome complement of 40 + 3 C. Considering that this single plant as well as all the 40 + C + Cil subcompactoids are secondary descendants from one single plant with a typical 40 + C + Cil behaviour, the interpretation of the »trisomic» karyotype as 40 + 3 C seems rather unlikely. The present writers think that the subcompactoid with 43 chromosomes can be given another interpretation, more adapted to genetical data, if the origin of subcompactoids is that suggested by SÁNCHEZ-MONGE and MAC KEY (1948). Thus, the »trisomic» subcompactoid in accordance with their hypothesis may be interpreted as a 40 + C + Cil + Cts type. Cts is here after HUSKINS (1946) used for the telomitic short arm of the C-chromosome. The 20 + Cil + Cts gamete is produced by misdivision of a C-univalent at meiosis followed by a non-disjunction of the two chromatids of the long arm, which will produce a Cil-chromosome. The suggested chromosome complement 40 + C + Cil + Cts will be further discussed in connection with the 0201 × 0715 strain (see below).

The genetic analysis of the normal subcompactoids is given in Table 3. The group »compactoids» is rather ambiguous as it may involve heterozygous plants of compactoid phenotype. For the heterogeneity test only normals, het speltoids and subcompactoids have been used.

Some anomalies, quite difficult to explain, have also been observed to occur in the 0715 speltoid strain. As the material during the whole genetical test has been sown in non-isolated plots, these anomalies may be explained by incrossing. This is probably the case with the both cytologically and genetically quite deviating plant, among others supplied to HUSKINS (1928). The progeny test speaks in favour of the explanation given by HUSKINS, i. e. that the deviating plants has been fertilized by *T. Spelta* pollen.

TABLE 3. *The progeny ratio af selfed normal subcompactoids (secondary mutation arisen in a B-series derived from the 0715 strain).*

Field number	No. of plant progenies tested	No. of progeny plants							No. of plants harvested in % of kernels sown
		normals	B het speltoids	B hom speltoids	subcompactoids	compactoids	chimaeras	total	
1928—425	17	128	89	—	141	6	—	364	64
1929—418	6	64	36	2	69	9	1	181	71
1930—425	15	381	223	6	293	22	—	925	42
1932—422	17	217	105	1	199	8	—	530	48
1935—452, 454	8	138	98	—	114	10	—	360	66
1937—951	5	104	60	—	84	3	—	251	57
1939—952	6	183	118	1	197	14	1	514	76
1941—952	7	102	59	—	134	4	—	299	58
1945—952	2	10	5	—	12	—	1	28	18
1947—963	5	145	97	—	172	8	—	422	57
Total	88	1472	890	10	1415	84	3	3874	56
Ratio		1 :	0,60 :	0,01 :	0,96 :	0,06 :	0,002		

Index of heterogeneity, $\chi^2 = 35{,}1378$; d. f. $= 18$; $0{,}01 > P > 0{,}001$

Another deviating ratio-type seems also difficult to explain. In a continuous ratio test of this sib the most common segregation type was found to be 1 : 3,5 : 1 of normals, het and hom speltoids, respectively. (28 plants of this type progeny tested.) But typical B het speltoids were also segregated out from this deviating strain. As the strain was discarded long ago, it is very difficult now to give an exact answer as to the cause of the high frequency of hom speltoids, characteristic of the deviating strain. The most feasible explanation seems, also here, to be the natural crossing (e. g. with a bearded normal or a bearded A het speltoid followed by a wrong classification of the hom speltoid group).

THE SPELTOID STRAIN FROM 0201 × 0715.

In the F_4, sown in 1917, of a cross between a purebred line of the German variety Emma (0201) and the 0715 strain of Börsum one het speltoid was observed in each of two of the lines. The progeny test gave 21 : 34 : 1 and 11 : 10 : 1 of beardless normals, beardless het speltoids and bearded hom speltoids, respectively. The continuous segregation test of the former family is given in Table 4. Here the normal, het speltoid and hom speltoid segregates have been used for the heterogeneity test.

The segregation behaviour is that of the C type, as this is described by NILSSON-EHLE (1921). The chromosome constitution of the strain proved very difficult to determine. Both HUSKINS (1927, 1928) and HÅKANSSON (1930) found rather contradictory results but both concluded (l. c.) that 43 chromosomes in the het speltoids was the most proper interpretation. HUSKINS, recently (1946, p. 492) discussing these chromosome counts, states, however, that »it now appears certain that these were either erroneous interpretations, or that extra chromosomes other than those affecting the speltoid complex were present». HÅKANSSON (l. c.) was quite near the correct interpretation that the characteristic complement at metaphase of C het speltoids is 20 normal bivalents and a heteromorphic bivalent composed of one normal C-chromosome and one C visibly deficient.

The occurrence of subcompactoids directly segregating out from series C het speltoids is quite unique (see Table 4). It has, however, been observed by NILSSON-EHLE (1921), though only in connection with a simultaneous change over from series B. Here in the present strain subcompactoids have arisen in the progeny of original C het speltoids of proved pedigree. Only one of the subcompactoids, that found in 1928—

TABLE 4. *The progeny ratio of selfed C het speltoids (original mutation arisen in the spring wheat cross 0201 × 0715).*

Field number	No. of plant progenies tested	No. of progeny plants							No. of plants harvested in % of kernels sown
		normals	C het speltoids	C hom speltoids	B het speltoids	subcompactoids	chimaeras	total	
1918—397	1	21	33	1	1	—	—	56	45
1919—500	5	78	73	4	—	—	—	155	41
1920—402	17	458	401	22	2	—	—	883	36
1921—195	9	118	165	10	—	—	—	293	57
1922—424	7	72	92	2	—	—	—	166	39
1923—444, 445	6	96	88	2	—	1	1	188	45
1924—444	22	340	345	15	—	—	—	700	57
1926—448, 449	21	964	1049	66	1	—	1	2081	58
1927—445	8	388	463	19	—	—	1	871	80
1928—421	16	627	600	67	—	1	—	1295	67
1929—419	13	304	307	21	—	—	—	632	56
1930—424, 426	16	733	884	52	1	5	—	1675	38
1932—423	4	188	207	16	—	—	—	411	44
Total	145	4387	4707	297	5	7	3	9406	53
Ratio		1 :	1,07 :	0,07 :	0,001 :	0,002 :	0,001		

Index of heterogeneity, $\chi^2 = 58{,}3485$; d. f. $= 22$; $P < 0{,}001$

421, has been carefully investigated. In its progeny 9 normals : 2 het speltoids : 5 subcompactoids were found. One of the het speltoids was then progeny-tested, showing a typical series C behaviour. The subcompactoid, therefore, must be assumed to have a chromosome complement of 40 + C + Cil + deficient C, i. e. about the same constitution as the one proposed for the above-mentioned subcompactoid of deviating karyotype, observed in the 0715 strain.

The phenotypic segregation of this subcompactoid type must be the same as that of the normal 40 + C + Cil type, as the deficient C-chromosome does not influence the speltoid and compactoid characters. 14 subcompactoids, all descendants of the 1928—421 plants, also gave a rather normal segregation, viz. a total progeny of 126 normals, 54 het speltoids, 4 hom speltoids, 97 subcompactoids and 5 problematic compactoids ($1 : 0{,}43 : 0{,}03 : 0{,}77 : 0{,}04$). Another two het speltoids were progeny-tested, showing the series C modus of segregation.

Secondary descendants (numbered 1930—426) of the 1928—421 subcompactoid have been cytologically investigated by HÅKANSSON (1931, 1932). Four plants had 43 chromosomes, three of these regularly forming a trivalent, one had 40 + C + Cil (in his own nomenclature 40 + no + co) and one had 40 + C + Cil + fragment. As the characteristic complement of the series C segregates was not known by HÅKANSSON, he interpreted the first-mentioned karyotype as 40 + 3 C and laid no stress on the fragment observed in the third type. In view of the present knowledge about the constitution of series C and the genetical data now gained it seems more probable that both his trisomic subcompactoids and the one with the fragment had the 40 + C + + Cil + deficient C set. His drawings do not contradict such a genetically more acceptable interpretation and concerning his trisomic subcompactoids he also confesses that »eingehender sind sie nicht untersucht» (1932, p. 177). When discussing the subcompactoid with the fragment he also states that »Chromosomenfragmente begegnet man ganz zufällig» (l. c., p. 176). His observation of the 40 + C + Cil subcompactoid and thus also B het speltoids in the secondary progeny of the 1928—421 subcompactoid is also quite in accordance with the segregation expected from a 40 + C + Cil + deficient C type.

As NILSSON-EHLE (1921) has already pointed out, the het speltoid progenies of C het speltoids often change to series B. He explained this as due to partial heterogamy and it has later been proved to be due to elimination of the deficient C-chromosome. In the present C-series B het speltoids have also been observed. The cases given in Table 4 are only

those discovered by the progeny test; probably this phenomenon is more frequent. Owing to lack of space the continuous progeny test will here only be given for one secondarily arisen B strain of 0201 × 0715, the one cytologically examined by SÁNCHEZ-MONGE and MAC KEY (1948; equal to their series II). This test is given in Table 5. The index of heterogeneity is based on the normal and het speltoid groups only.

TABLE 5. *The progeny ratio of selfed B het speltoids (secondary mutation arisen in a C-series derived from the cross 0201 × 0715).*

Field number	No. of plant progenies tested	No. of progeny plants					No. of plants harvested in % of kernels sown
		normals	B het speltoids	B hom speltoids	subcompactoids	total	
1932—423	1	10	70	2	1	83	36
1935—455	5	48	223	3	4	278	51
1937—952	5	10	178	—	—	188	54
1939—953	4	42	304	1	2	349	53
1941—953	7	9	166	1	—	176	32
1945—953	4	21	45	—	1	67	13
1947—964	4	17	106	1	—	124	59
Total	30	157	1092	8	8	1265	43
Ratio		1 :	6,96 :	0,05 :	0,05		

Index of heterogeneity, $\chi^2 = 46{,}7819$; d. f. $= 6$; $P < 0{,}001$

From Table 5 it is evident that this secondarily arisen speltoid strain has a typical series B behaviour. This has also been cytologically proved by the above-mentioned workers. They found 40 + C in the het speltoids and 40 + C + Cil in the subcompactoids. The latter type was found in quite a high frequency (0,63 %) in the progeny of het speltoids. 19 subcompactoids tested gave together 207 normals, 116 B het speltoids, 2 B hom speltoids, 191 subcompactoids and 15 compactoids or 1 : 0,56 : 0,01 : 0,92 : 0,07, respectively, in the progeny (heterogeneity between 6 years of testing: $\chi^2 = 31{,}1767$; d. f. $= 10$; $P < 0{,}001$). The genetical analysis, thus, confirms the prediction suggested by the cytological investigation.

THE SPELTOID STRAIN FROM 0750 × BÖRSUM.

In the preliminary yield trial of 1917 a het speltoid plant was observed in an F_4 line of the cross 0750 (a purebred line of the above-mentioned Emma marketed under the name of Vårpärl) × Börsum.

As the progeny test of this plant gave nearly the same result as that of the 0201 × 0715 strain, it will be only briefly reported. The ratio-type of the original series C is, however, as is clear from Table 6 here characterized by a higher zygotic elimination of hom speltoids. Thus, the test of heterogeneity could only be based on the beardless normal and het speltoid groups. Subcompactoids and chimaeras have not been observed.

The change from series C to series B het speltoids in the present strain has been discovered to be in about the same frequency as in the

TABLE 6. *The progeny ratio of selfed C het speltoids (original mutation arisen in the spring wheat cross of 0750 × Börsum).*

Field number	No. of plant progenies tested	No. of progeny plants				No. of plants harvested in % of kernels sown
		normals	C het speltoids	C hom speltoids	B het speltoids	total	
1918—408	1	6	13	—	—	19	?
1919—502	4	50	68	—	2	120	62
1920—404	20	582	678	2	—	1262	56
1922—425	19	304	363	12	—	679	44
1923—430	37	890	809	11	—	1710	52
1926—439	25	614	636	4	3	1257	41
1927—434—437	41	1839	1929	31	—	3799	79
1928—422	4	134	167	4	1	306	75
1929—420	4	114	141	3	—	258	57
1930—427	5	262	279	2	—	543	47
Total	160	4795	5083	69	6	9953	57
Ratio		1	: 1,06	: 0,01	: 0,001		

Index of heterogeneity, $\chi^2 = 23{,}4522$; d. f. $= 9$; $0{,}01 > P > 0{,}001$

0201 × 0715 strain (± 0,1 %). Typical of all the secondary series B obtained from the 0750 × Börsum strain is the pronounced excess of het speltoids. One of these B-series, that given in Table 7, has been cytologically studied by SANCHEZ-MONGE and MAC KEY (1948; equal to their series III). They found a typical series B behaviour and a very frequent formation of micronuclei in the tetrads (l. c., Table 4), which gives a good explanation both of the high frequency of het speltoids and of the very low frequency (0,11 %) of subcompactoids in the progeny of the selfed het speltoids.

The subcompactoids from the present series B seem in at least by far the greater part to be of the common 40 + C + Cil type. Their segregation behaviour is given in Table 8. The test of heterogeneity is only based on the three main groups of segregates, the »compactoid»

group also here being a very problematic group. Owing to their phenotype several plants have been classified as compactoids but the next year have been found to give a heterotypic progeny. In several cases plants with normal ears and straw height have been found besides the short and dense-eared offspring. Not until recently has special attention been paid to this observation. The »normal» plants, thus, have never been progeny-tested. They may have the 40 + Cil constitution and be

TABLE 7. *The progeny ratio of selfed B het speltoids (secondary mutation arisen in a C-series derived from the cross 0750 × Börsum).*

Field number	No. of plant progenies tested	No. of progeny plants					No. of plants harvested in % of kernels sown
		normals	B het speltoids	B hom speltoids	subcompactoids	total	
1920—404	1	8	93	—	—	101	55
1922—425	7	20	180	4	—	204	39
1923—430	30	70	463	1	—	534	35
1926—439	10	43	338	—	—	381	31
1927—434	19	144	1631	4	2	1781	68
1928—422	20	59	757	14	2	832	68
1929—420	9	22	150	4	—	176	63
1932—424	5	42	326	4	2	374	52
1935—456	5	23	242	1	—	266	54
1937—953	5	60	572	4	—	636	64
1939—954	3	13	168	3	—	184	52
1941—954	4	17	286	7	—	310	52
1945—954	5	26	71	—	—	97	17
1947—965	5	169	580	5	1	755	70
Total	128	716	5857	51	7	6631	51
Ratio		1	: 8,18	: 0,07	: 0,01		

Index of heterogeneity, $\chi^2 = 174{,}3425$; d. f. = 13; $P < 0{,}001$

identical with the subnormal type observed by other speltoid workers. In the present strain as well as in the 0715 strain they must, if produced, have just the same phenotype as the 40 + 2 C plants.

TWO SPELTOID STRAINS FROM INDIGENOUS SPRING WHEAT VARIETIES.

As basic material for the wheat breeding at Svalöf the Wheat and Oat Department has collected a large number of old indigenous varieties. These are sown every two years in small space-planted plots and kept pure by a continuous control. In 1927 this control disclosed one het

TABLE 8. *The progeny ratio of selfed normal subcompactoids (tertiary mutation arisen in a B-series of 0750 × Börsum origin).*

Field number	No. of plant progenies tested	No. of progeny plants: normals	B het speltoids	B hom speltoids	subcompactoids	compactoids	total	No. of plants harvested in % of kernels sown
1929—420	1	10	1	—	6	—	17	41
1930—427	3	49	15	—	52	2	118	49
1932—424	6	95	26	—	116	9	246	37
1935—456	15	250	93	—	257	15	615	57
1937—953	5	112	62	1	81	—	256	61
1939—954	5	116	43	—	153	7	319	50
1941—954	5	77	48	—	114	3	242	58
1945—954	3	84	43	—	66	—	193	70
1947—965	5	48	23	—	74	—	145	73
Total	48	841	354	1	919	36	2151	56
Ratio		1	: 0,42	: 0,001	: 1,09	: 0,04		

Index of heterogeneity, $\chi^2 = 48{,}8610$; d. f. $= 14$; $P < 0{,}001$

speltoid in a wheat from the province of Halland and one in a wheat from the province of Dalarne. The two old varieties must be supposed to have a very high degree of homozygosity. Consequently, there was reason to believe that the speltlike habit was the only heterozygous character involved and from this point of view the two het speltoids were found worth preserving.

TABLE 9. *The progeny ratio of selfed C het speltoids (original mutation arisen in the Halland spring wheat).*

Field number	No. of plant progenies tested	No. of progeny plants: normals	C het speltoids	C hom speltoids	total	No. of plants harvested in % of kernels sown
1928—417	1	7	4	—	11	100
1929—423	2	145	141	11	297	75
1930—428	5	231	293	30	554	36
1932—425	3	165	146	6	317	44
1935—457	3	98	100	9	207	64
1937—954	3	191	160	12	363	64
1939—955	3	135	148	11	294	60
1941—955	3	118	78	6	202	58
1945—955	3	34	37	1	72	15
1947—966	5	305	302	16	623	65
Total	31	1429	1409	102	2940	54
Ratio		1	: 0,99	: 0,07		

Index of heterogeneity, $\chi^2 = 31{,}4494$; d. f. $= 14$; $0{,}01 > P > 0{,}001$

The progeny test (Tables 9 and 10) showed them both to be of the series C type, very constantly only segregating in normals, C het and C hom speltoids. As the Dala wheat is bearded, all the segregates were bearded, in the Halland strain only the hom speltoids. The Dala strain has been cytologically studied by HÅKANSSON (1930). At metaphase I of the het speltoid he observed 20 normal bivalents and a heteromorphic configuration which he first interpreted as a bivalent, later as a trivalent. His first impression now appears to have been correct, especially as he found 42 chromosomes to be the most characteristic number of the hom speltoid. The Dala strain, thus, probably has the normal series C set, 40 + C + deficient C.

TABLE 10. *The progeny ratio of selfed C het speltoids (original mutation arisen in the Dala spring wheat).*

Field number	No. of plant progenies tested	No. of progeny plants				No. of plants harvested in % of kernels sown
		normals	C het speltoids	C hom speltoids	total	
1928—417	1	30	28	4	62	86
1929—424	3	125	127	9	261	59
1930—428	11	379	487	39	905	40
1932—426	3	145	174	13	332	34
1935—458	3	126	112	1	239	60
1937—955	3	91	123	1	215	53
1939—956	3	149	156	13	318	58
1941—956	3	107	94	5	206	63
1945—956	3	33	32	2	67	14
1947—967	5	266	328	12	606	76
Total	38	1451	1661	99	3211	51
Ratio		1	: 1,14	: 0,07		

Index of heterogeneity, $\chi^2 = 34{,}9007$; d. f. $= 14$; $0{,}01 > P > 0{,}001$

GENERAL CONCLUSIONS.

The different het speltoid ratio-types observed may be regarded as due to three fundamental factors, the elimination of C-chromosomes at meiosis (only decisive for series B), the lower functioning ability of the speltoid-bearing pollen (obvious in all the three segregation types though of different degrees) and the directed elimination of zygotes (ditto).

Series A and C are not distinctly separated as to their modus of segregation. They run into each other, while there is a rather well-defined step down to the loss of a whole chromosome, characteristic of B het speltoids.

The degree of zygotic elimination runs parallel with the extent of substance loss from the C-chromosome. In the heterozygous state there is, however, only a very slight decrease in the surviving ability. The elimination here certainly occurs more than to a normal extent, but in the present spring-sown material this is only significant in the B-series ($0{,}05 > P > 0{,}02$) if the number of plants harvested in per cent of kernels sown is a representative measure of the phenomenon. This experience is in good correspondence to the germination tests performed by UCHIKAWA (1941).

The elimination of hom speltoid zygotes is, however, very pronounced. Compared with normal seed UCHIKAWA (l. c.) found 4, 15 and 18 % lower germinability of seed taken from A, C and B hom speltoids, respectively. The corresponding figures, based on the number of plants harvested in per cent of kernels sown, are in the present investigation 8, 11 and 39 %. The last-mentioned mode of estimating the zygotic elimination seems quite satisfactory, as abortion does not play any important rôle, as already shown by NILSSON-EHLE (1921). Among 10212 florets from normal plants he found a seed-setting ability of 93,7 %, among 1797 florets from B het speltoids only about one per cent lower, or 92,6 %.

The degree of failure of the speltoid-bearing pollen to function at the fertilization also corresponds quite well with the changes in the C-chromosome. From cross experiments ÅKERMAN (1923) found the normal pollen about 4 times as effective at the fertilization as the speltoid-bearing pollen of series A. The corresponding figures given by UCHIKAWA (1941) show a 10 times greater pollen effectivity in series C and a 20 times greater in series B. In view of the fact that an elimination of C-univalents at meiosis of B het speltoids causes the 20-chromosome pollen to be 4 times more frequent than the 20 + C type, the estimated certation between normal and speltoid-bearing pollen will give a sufficient explanation of the normal: het speltoid ratio characteristic to series A, C and B, respectively. The frequency of zygotes produced by selfed het speltoids will thus be 1 normal : 1,25 het speltoid : 0,25 hom speltoid for the A type, 1 : 1,1 : 0,1 for the C type and 1 : 4,2 : 0,8 for the B type (cf. UCHIKAWA, l. c.). For the series A and C these estimated frequencies are quite in agreement with the observed data based on mature plants. The zygotic elimination estimated above is enough to explain the small deficit often found in the hom speltoid group. In the B-series the elimination of hom speltoid zygotes must, however, occur in 80—90 per cent if the zygotic ratio given above is

correctly determined. The estimation, based on the 0715 strain, of the pollen certation in series B given in the present paper seems, therefore, more fitted to the actual data. Here, the normal pollen was shown to be 80 times as effective as the speltoid-bearing pollen at the fertilization, thus giving a zygotic frequency of 1 : 4,05 : 0,2. The varying degree in the excess of het speltoids in the ratio-types of the B strains may be explained by the more or less frequent elimination of C-univalents at meiosis.

The varying competitive ability of the speltoid-bearing pollen, however, just as the variation in the zygotic elimination, is not dependent only on the genetic constitution. Environmental factors also play an important rôle, the more so the weaker the pollen is. This fact is well illustrated by the segregation series of the present paper. A heterogeneity test based only on the more vital normal and het speltoid segregates gives $P > 0{,}50$ for the A-strain, $P > 0{,}001$, $P > 0{,}001$, $P > 0{,}01$ and $P > 0{,}05$ for the four C-strains examined, and $P < 0{,}001$ for each of the three B-strains. Though not quite comparable (number of years and number of progenies per year varying) the P-values give a good indication of the parallelism between environmental influence and the frailty of the pollen. Attempts have been made to correlate the het speltoid: normal ratios with meteorological data from the days around the heading of the preceding year. Although rather wide variations exist within the material, there seems to be a trend of a higher degree of functioning of the speltoid-bearing pollen under weather conditions favouring a rapid flowering.

SUMMARY.

The paper gives a genetical analysis of six speltoid strains derived from spring-sown *Triticum vulgare*. As the strains have been cytologically investigated by several workers (WINGE, HUSKINS, HÅKANSSON, and SÁNCHEZ-MONGE and MAC KEY), their interpretations have been discussed in the light of the genetical data.

Subcompactoids as direct segregates from original C het speltoids have been observed. From genetical data they are considered to have a chromosome constitution of 40 + C + Cil + Cts (Cil = iso-chromosome composed of the duplicated long arm of a C-chromosome; Cts = telomitic short arm of a C-chromosome). Such an inpretation is not contradicted by the cytological studies.

Cross experiments performed by ÅKERMAN (1923) and UCHIKAWA

(1941) have been confirmed or corrected. Together with data collected about the zygotic elimination these results have been used to explain the characteristic ratio-types of series A, B and C. A pronounced parallelism between the frailty of the speltoid-bearing pollen, the elimination of zygotes and the sensibility to environmental factors on one side and the extent of substance loss from the C-chromosome on the other has been found.

LITERATURE CITED.

1. HUSKINS, C. L. 1927. Genetical and cytological studies of fatuoid oats and speltoid wheats. — Proc. V. Int. Gen. Congr. (Zschr. f. ind. Abst.- u. Vererb., Suppl. 1, pp. 907—916).
2. — 1928. On the cytology of speltoid wheats in relation to their origin and genetic behaviour. — Journ. of Genetics, Vol. 20, pp. 103—122.
3. — 1946. Fatuoid, speltoid and related mutations of oats and wheat. — Bot. Rev., Vol. 12, pp. 457—514.
4. HÅKANSSON, A. 1930. Zytologische Beobachtungen an s. g. Speltoidheterozygoten beim Weizen. — Sv. Bot. Tidskr., Vol. 24: 1, pp. 44—57.
5. — 1931. Die Chromosomenzahl von Speltoidheterozygoten, die aus s. g. subcompactum-Typen beim Weizen hervorgegangen sind. — Botaniska Notiser, 1931, pp. 343—345.
6. — 1932. Zytologische Studien an compactoiden Typen von *Triticum vulgare*. — Hereditas, Vol. XVII, pp. 155—196.
7. NILSSON-EHLE, H. 1917. Untersuchungen über Speltoidmutationen beim Weizen. — Botaniska Notiser, 1917, pp. 305—329.
8. — 1920. Multiple Allelomorphe und Komplexmutationen beim Weizen. — Hereditas, Vol. I, pp. 277—311.
9. — 1921. Über mutmassliche partielle Heterogamie bei den Speltoidmutationen des Weizens. — Hereditas, Vol. II, pp. 25—76.
10. NISHIYAMA, I. 1931. The genetics and cytology of certain cereals. II. Karyogenetic studies of fatuoid oats with special reference to their origin. — Jap. Journ. of Genetics, Vol. 7, pp. 49—101.
11. SÁNCHEZ-MONGE, E. and MAC KEY, J. 1948. On the origin of subcompactoids in *Triticum vulgare*. — Hereditas, Vol. XXXIV, pp. 321—337.
12. UCHIKAWA, I. 1941. Genetic and cytological studies of speltoid wheat. II. Origin of speltoid wheat. — Mem. Coll. Agric. Kyoto Imp. Univ., 50, pp. 1—64.
13. WINGE, Ö. 1924. Zytologische Untersuchungen über speltoide und andere mutantenähnliche Aberranten beim Weizen. — Hereditas, Vol. V, pp. 241—286.
14. ÅKERMAN, Å. 1920. Speltlike bud-sports in common wheat. — Hereditas, Vol. I, pp. 116—127.
15. — 1923. Beiträge zur Kenntnis der Speltoidmutationen des Weizens. I. Untersuchungen über eine Speltoidform aus schwedischem Sammetweizen. — Hereditas, Vol. IV, pp. 111—124.

16. ÅKERMAN, Å. 1927. Weitere Studien über Speltoidchimären bei *Triticum vulgare*. — Hereditas, Vol. IX, pp. 321—334.

CONTENTS.

The speltoid strain from Brown Schlanstedt × 0715 302
The speltoid strain from 0715 304
The speltoid strain from 0201 × 0715 309
The speltoid strain from 0750 × Börsum 312
Two speltoid strains from indigenous spring wheat varieties 314
General conclusions 316
Summary 318
Literature cited 319

Communications from the Swedish Seed Association. No. 14.

ON THE ORIGIN OF SUBCOMPACTOIDS IN TRITICUM VULGARE

BY *E. SÁNCHEZ-MONGE* AND *J. MAC KEY*
WHEAT AND OAT DEPARTMENT OF THE SWEDISH SEED ASSOCIATION, SVALÖF

IN 1904 NILSSON-EHLE found among the progeny of »squarehead» *Triticum vulgare* HOST. several plants resembling *Tr. spelta* L. in their ear shape. He called them speltoids and showed conclusively (1917, 1920, 1921) that they arise through mutation. He was also the first to observe another aberrant form, resembling *Tr. compactum* HOST., the subcompactum or subcompactoid type. Since then, a great number of investigators (VESTERGAARD, ÅKERMAN, LATHOUWERS, LINDHARD, KAJANUS, WINGE, GOULDEN, HUSKINS, WATKINS, PHILIPTSCHENKO, VASILIEV, HÅKANSSON, MÜNTZING, OEHLER, DUMON, PHIPPS and GUERNEY, NISHIYAMA, ISHIKAWA, UCHIKAWA, CAMARA, SMITH, MATSUMURA and LOVE; for complete bibliography, see HUSKINS, 1946) have studied the genetics and cytology of these off-types in order to ascertain their origin and relationship.

NILSSON-EHLE (1921) classified the speltoids into three serial types. According to the segregation ratio of the heterozygotes when self-fertilized, his series can be characterized thus:

Series	Normals	Het speltoids	Hom speltoids
A	1 :	< 2 :	< 1
B	1 :	4—5 :	$< 0{,}1$
C	1 :	1 :	$< 0{,}2$

Series C rather often changes over to series B. Series A and B are more stable in this respect.

Subcompactoids generally arise as segregates of het B speltoids. Sometimes they appear in series C and also directly in normal *Tr. vulgare*. Subcompactoids are heterozygous but owing to their different genetic origin the segregation is not uniform. HUSKINS (1946) distinguishes three main segregation types. One type gives only normal, subcompactoid and true-breeding compactoid progeny, another het speltoids and a third type het speltoids and subnormals in addition.

WINGE, GOULDEN and HUSKINS discovered that several of these off-

types showed irregularities in chromosome number and behaviour and were the first to establish the relationship between chromosome irregularities, origin and genetic behaviour in these mutants. Thanks to the work of the investigators mentioned and others, the origin of the three series of speltoid wheats seems definitively explained.

HUSKINS summarizes in a recent paper (1946) the investigations concerning the speltoid problem and concludes that:

Series A arises by a more or less complex gene mutation or by an undetectable deficiency in a C-chromosome.

Series B arises by a loss of a C-chromosome.

Series C arises by a visible deficiency in a C-chromosome.

The C-chromosome carries the inhibitors of the genes for bearded and speltoid, situated in the B-chromosome. The C-chromosome has a submedian centromere, and following HUSKINS's nomenclature we use in this paper:

C for the normal chromosome.

Cd for the C-chromosome with an appreciable deficiency.

Cts for the telomitic short arm of the C-chromosome.

Ctl for the telomitic long arm of the C-chromosome.

Cil for the iso-chromosome composed of a duplicated longer arm of the C-chromosome.

As HUSKINS (l. c.) summarizes, the chromosome complements of the different types found in the speltoid series are the following:

Normal	(40 + 2 C).
B het speltoid	(40 + C) or (40 + Ctl).
B hom speltoid	(40).
C het speltoid	(40 + C + Cd) or (40 + C + Cts).
C hom speltoid	(40 + 2 Cd) or (40 + 2 Cts).
Subnormal	(40 + C + Ctl) or (40 + 2 Ctl) or (40 + Cil).
Subcompactoid	(40 + 3 C) or (40 + C + Cil) or (40 + Ctl + + Cil) or (40 + 2 C + Cil).
Compactoid	(40 + 4 C) or (40 + 2 Cil) or (40 + C + 2 Cil) or (40 + 2 C + 2 Cil).

Thus, less than two sets of genes suppressing the speltoid and the bearded characters will cause a speltoid phenotype, while more than two sets will give rise to compactoid types. As the inhibitors are situated in the longer arm of the C-chromosome, it may be observed that the

Cts-chromosome does not influence the speltoid and compactoid characters.

In spite of the extensive work already carried out in order to account for the speltoid and compactoid mutations these problems cannot be regarded as definitely solved. In the present paper special attention is paid to the origin of subcompactoids. Earlier investigations of this were mainly concentrated upon the subcompactoid as a type. The present one is an attempt to give an explanation of this problem by studying the behaviour of the C-univalent during meiosis in het speltoids of the B-series from which subcompactoids rather frequently segregate. Special attention is devoted to the second meiotic division and the formation of micronuclei in the tetrads.

The material used in the present studies consists of three spring wheat series of B speltoids, kindly placed at our disposal by Professor Å. ÅKERMAN. Series I originates from a het speltoid that he found in a purebred line of Börsum. Series II and III are B-types that have arisen through a second mutation in two C-series isolated in 1917 from the crosses 0201 × 0715 and 0750 × Börsum, respectively. A genetic analysis of the three B-series is given by ÅKERMAN and MAC KEY (1948).

The anthers were fixed in CARNOY (6 : 3 : 1). Permanent smears of the anthers stained in FEULGEN were made according to the technique of HILLARY (1940).

The authors' sincere acknowledgements are due to Dr. A. LEVAN for the facilities given and for much useful advice. We are also indebted to Mr. JOE HIN TJIO for his help in taking the microphotographs.

I. OBSERVATIONS IN PMC'S OF HET B SPELTOIDS.

Metaphase I. — Four types of chromosome association were observed, namely

20 bivalents + 1 univalent (Figs. 1, 7, 35, 36).
19 bivalents + 3 univalents (Figs. 3, 8).
19 bivalents + 1 trivalent (Figs. 2, 9, 37).
18 bivalents + 1 tetravalent + 1 univalent (Fig. 4).

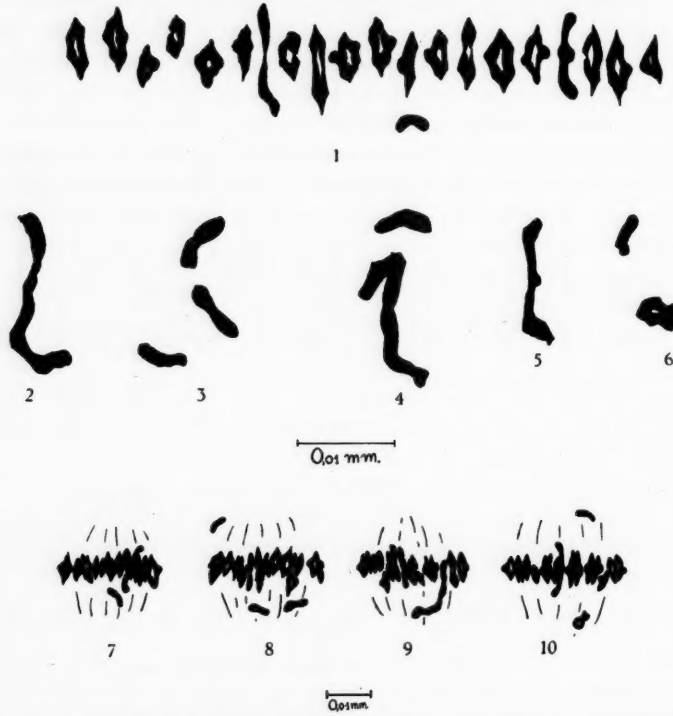
The observed frequencies are given in Table 1.

The univalents generally lie outside the plates.

Anaphase I. — In all plates observed of the three series the bivalents separate normally, while the univalent remains as a laggard and splits longitudinally at late anaphase (Figs. 11, 39).

TABLE 1. *Chromosome association at metaphase I of B het speltoids.*

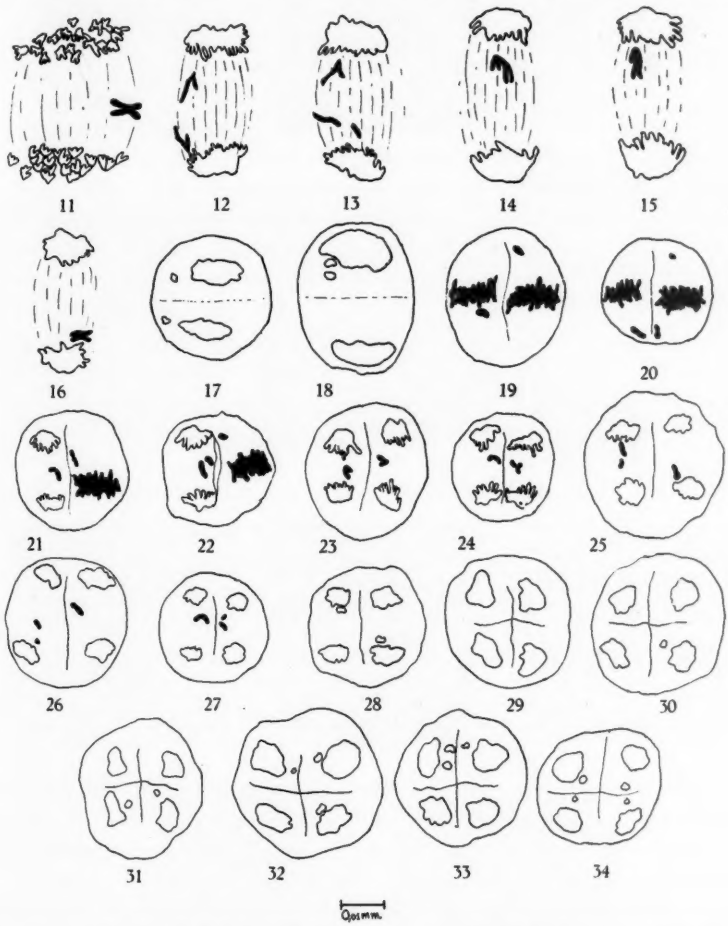
Series	Number of PMC's with			
	$20_{II}+1_{I}$	$19_{II}+3_{I}$	$19_{II}+1_{III}$	$18_{II}+1_{IV}+1_{I}$
I	317	6	2	—
II	318	—	5	1
III	276	5	1	—
Total	911	11	8	1



Figs. 1—4, metaphase I of B het speltoids. — Fig. 1, chromosome complement. — Fig. 2, trivalent, including the C-chromosome. — Fig. 3, three univalents in the same plate. — Fig. 4, a tetravalent and a univalent in the same plate. — Figs. 5—6, metaphase I of subcompactoids. — Fig. 5, heteromorphic bivalent. — Fig. 6, the Cil- and C-univalents. — Figs. 7—9, metaphase I of B het speltoids (not all the bivalents are visible). — Fig. 7, $20_{II}+1_{I}$. — Fig. 8, $19_{II}+3_{I}$. — Fig. 9, $19_{II}+1_{III}$. — Fig. 10, metaphase I of subcompactoid, showing 20 + C + Cil.

Telophase I. — Generally the two chromatids of the C-univalent pass each to its pole (Fig. 12) and are often included in the daughter nuclei (Fig. 40).

In three cases out of 119 observations the two univalent-halves were seen moving, apparently without complete separation, to the same pole



Figs. 11—34, PMC's of B het speltoids. — Fig. 11, splitting of the C-univalent at anaphase I. — Fig. 12, normal telophase I. — Fig. 13, misdivision of the C-univalent at telophase I. — Figs. 14—16, non-disjunction of the univalent-halves at telophase I. — Figs. 17—18, micronucleus distribution in dyad cells. — Fig. 19, normal metaphase II. — Fig. 20, metaphase II showing a misdivided C-univalent. — Fig. 21, C-halves at metaphase II and anaphase II. — Fig. 22, telophase II showing a misdivided C-univalent. — Fig. 23, normal telophase II. — Figs. 24—27, misdivision at telophase II. — Fig. 28, formation of micronuclei at late telophase II. — Figs. 29—34, tetrads showing different possibilities for micronucleus formation.

(Figs. 14, 15) and in one case two quite separated halves were observed going together to the same pole (Fig. 16).

In two cases one of the two separated halves of the univalent was broken at the centromere, a misdivision having evidently occurred.

Interkinesis. — Interkinesis with 0, 1 or 2 micronuclei (Figs. 17, 41) was observed, according to the number of split halves of the univalent not included in the daughter nuclei. Corresponding with the anomalous cases found at first telophase, two micronuclei included in the same daughter cell were also observed at interkinesis. This may be due to the arrival of the two halves of the univalent at the same pole, but fragmentation of one of the halves is also a possible explanation. No obvious difference could, however, be seen in the size of the two micronuclei (Fig. 18).

In one case 1 micronucleus was observed at one pole and 2 at the other, one of the latter being distinctly smaller than the other. This case must obviously be due to the fragmentation observed at telophase I.

The observed frequencies of micronuclei are given in Table 2.

TABLE 2. *Formation and distribution of micronuclei at the interkinesis of B het speltoids.*

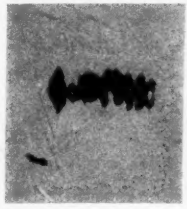
Series	Number of micronuclei at interkinesis				
	0 + 0	0 + 1	1 + 1	0 + 2	1 + 2
I	62	43	42	2	—
II	31	14	13	1	—
III	34	32	85	—	1
Total	127	89	140	3	1

Metaphase II. — The univalent frequently appears as a micronucleus at early metaphase (Fig. 19), and as the metaphase advances it takes the characteristic form of a chromosome with submedian constriction (Fig. 21).

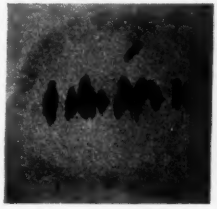
In four cases out of 103 observations the two halves of the original univalent appear in the same dyad cell. This is the logical consequence of the type of first metaphase in which the two halves pass to the same pole. Only in one case was one of the univalent halves fragmented (Fig. 20). This misdivision evidently occurred at the first telophase.

Anaphase II. — The univalent has the same appearance as in late metaphase and remains as a laggard at late anaphase.

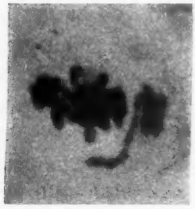
Telophase II. — At this stage the univalent is observed either to be



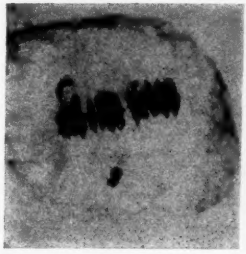
35



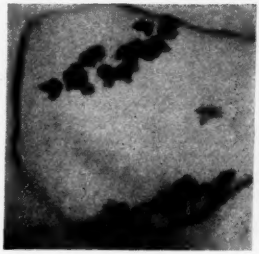
36



37

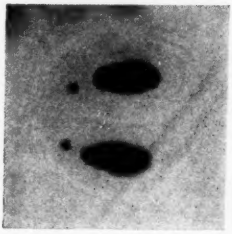


38

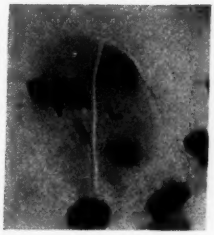


39

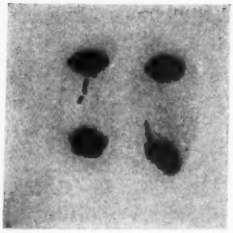
40



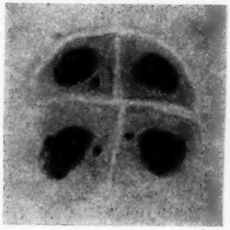
41



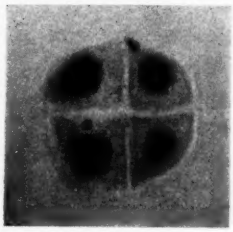
42



43



44



45

Figs. 35—37, metaphase I of B het speltoids. — Figs. 35—36, $20_{II} + 1_{I}$. — Fig. 37, $19_{II} + 1_{III}$. — Fig. 38, metaphase I of subcompactoid showing $20_{II} + C + Cil$. — Figs. 39—45, B het speltoids. — Fig. 39, normal anaphase I. — Fig. 40, normal telophase I. — Fig. 41, normal interkinesis. — Fig. 42, C-halves at metaphase II and anaphase II. — Fig. 43, misdivision at telophase II. — Figs. 44—45, tetrads showing exclusion of the misdivided C-chromosome.

included in a daughter nucleus or to form a micronucleus (Figs. 21, 23, 42). At late telophase inclusion of the two halves was observed in 21 cases, of one of the halves in 11 cases and of neither of the halves in 74 cases. Among the last-mentioned cases misdivision of one of the halves was observed in 17 cases (Figs. 22, 24—27, 43). This high frequency cannot be explained as a consequence of the misdivision found at first telophase. From Table 3 it is clear that this phenomenon also must occur at second telophase.

TABLE 3. *The frequencies of misdivision observed at telophase I and II in B het speltoids.*

Number of PMC's with	Telophase I	Telophase II
Normally divided C-chromosomes	117	89
Misdivided C-chromosomes	2	17
Total	119	106
Misdivision, %	1,7	16,9

Tetrads. — In the tetrads 0 to 4 micronuclei were observed (Figs. 29—34). When there were three micronuclei in the tetrads, one of them was always smaller than the others (Figs. 32, 33, 44, 45), and when there were four micronuclei, two of them were smaller than the others (Fig. 34), all in accordance with observations in earlier stages.

The found frequencies and the distribution of micronuclei in tetrads are given in Table 4.

TABLE 4. *Formation and distribution of micronuclei in tetrads of B het speltoids.*

Series	Number of micronuclei in the tetrads						
	0+0+0+0	0+0+0+1	0+0+1+1	0+1+1+1	1+1+1+1	0+0+0+2	0+0+1+2
I	54	25	25	6	—	—	—
II	26	11	9	1	—	1	—
III	94	73	155	21	3	5	3
Total	174	109	189	28	3	6	3

II. OBSERVATIONS IN PMC'S OF SUBCOMPACTOIDS.

Cytological studies of subcompactoids have unveiled a univalent able to form a chiasma between its two arms (HÅKANSSON's co-chromosome, HUSKINS's Cil-chromosome). In order to check whether this phenomenon was also present in our material the meiosis was studied

of subcompactoids of the same pedigree as the examined het B speltoids. The characteristic chiasma formation of the Cil-chromosome was frequently observed (Figs. 6, 10, 38). Sometimes the Cil-chromosome was observed forming a heteromorphic bivalent together with the C-chromosome (Fig. 5). Observations of chromosome association at first metaphase gave the following frequencies:

$20_{II} + C + Cil$	60	cases
20_{II} + heteromorphic bivalent	2	»
$19_{II} + 1_{III} + Cil$	2	»

The examination thus shows that our subcompactoids belong to the same type as the »normaler Subcompactum», described by HÅKANSSON (1932).

III. DISCUSSION.

Our observations concerning the different types of chromosome association at metaphase I, with $20_{II} + 1_{I}$ as the most common conjugation, are in complete agreement with the observations of other authors. The percentage of irregularities observed varies from 1,9 to 2,5 %. These figures are higher than those given by UCHIKAWA (1941) for his B-series, where he found 0,63 to 0,79 % of irregularities. Considering, for instance, that HOLLINGSHEAD (1932) has found 2,9 to 9,7 % of metaphase plates with univalents in the first meiotic division of PMC's of 5 varieties of *Tr. vulgare* and 5,2 to 39,1 % in 10 of their hybrids, we think that our material represents a quite normal behaviour.

As MÜNTZING (1930) and UCHIKAWA (1941) have already suggested, the origin of the trisomic subcompactoid type (40 + 3 C) can be explained by non-disjunction at first telophase. Thus the two C-univalent halves will be included in the same dyad cell and if they are distributed at random during the second division, as is stated by UCHIKAWA, germ-cells with 20 + 2 C chromosomes may be formed. This interpretation is quite in harmony with the observations made by the present writers. The low frequency of trisomic subcompactoids found as segregates from speltoid series may be explained by the rare cases of non-disjunction, the certainly low possibility of the two daughter-univalents being included in the same tetrad nucleus and the necessary fertilization with a 20 + C gamete.

Attempts to explain the origin of the more common 40 + C + Cil subcompactoid type have also been made. HÅKANSSON (1932) suggested

that the Cil-chromosome (in his own nomenclature called the co-chromosome) may arise through crossing over between the two arms of the C univalent in B het speltoids. Thus, the proximal part of the chromosome arms is thought to be unchanged, while the distal part of the arms must be identical. Postulating the speltoid-inhibiting genes situated in the distal part of the longer C-chromosome arm, the 40 + + C + Cil plants will have three such gene sets and, therefore, behave as subcompactoids. In order to explain the chiasma formation between the two arms of the C-chromosome a duplication is supposed to exist. The cytological observations of B het speltoids argue, however, hardly in favour of such a mechanism, at least not as the most common one. HÅKANSSON emphasizes that he has never observed any clear evidence of segmental duplication within the C-chromosome. HUSKINS and SMITH (unpublished; cf. HUSKINS, 1946) seem to be the only authors who have found that the single C-chromosome in B het speltoids is able to pair on itself. Nor does the figuration of the heteromorphic C + Cil bivalent, observed in subcompactoids by several authors, seem in complete agreement with the duplication hypothesis as a universal explanation. According to this hypothesis the Cil-chromosome must be capable of homologous pairing with the C-univalent not only along the whole length of one arm but also along the proximal part of the other. This makes the figuration <>< possible. As both HÅKANSSON (1932) and the present writers (Fig. 5) have observed only C + Cil bivalents with one chiasma, the hypothesis given in the following discussion seems more adapted to the cytological data. The genetic consequence will, however, be the same for the two explanations.

The origin of the Cil-chromosome is presumed to be primarily caused by misdivision of the C-univalent in B het speltoids. This phenomenon has not previously been clearly pointed out in speltoid wheat (see, however, HÅKANSSON, 1930, Fig. 5) but has been observed in the related fatuoid mutations in oats; NISHIYAMA (1931) has found fragmentation of the univalent at second telophase. Without knowing the occurrence of misdivision, he stated that »the fragmentation appeared usually to occur at a point where the spindle fibres are attached» (l. c., p. 73). Other cases of transverse division of the centromere were observed by UPCOTT (1937), by KOLLER (1938) in *Pisum* and by DARLINGTON (1939) in *Fritillaria*. Misdivision has further been found by HÅKANSSON (1940 a, b) in a haploid plant of *Godetia Whitneyi* and in a mutant of the same species. New telocentric chromosomes produced by misdivision were observed by LEVAN (1942) in the second

meiotic division of a haploid rye. MÜNTZING (1944) stated that certain extra fragment chromosomes in rye are produced by misdivision. The chromosomes with terminal centromere found in *Triticum* by HUSKINS and SPIER (1934), LOVE (1938), and HUSKINS and SMITH (l. c.) may also be explained as a product of misdivision.

The misdivision observed at first telophase of B het speltoids seems to be quite rare. It can, however, be traced from telophase I (1,7 %) over interkinesis (0,3 %) to metaphase II (1,0 %). As the phenomenon was observed in two of the three series (II and III), it cannot be described as unique. The frequent occurrence of misdivision at telophase II cannot, however, as stated above, be explained only as due to the fragmentation observed at telophase I. A frequency of 16,0 % misdivided C-univalents (or 8,0 % of split chromatid centromeres) observed at telophase II and an elimination of 6,2 % (or 3,6 %, respectively) of these fragments in the tetrads must indicate that the second division is the most important source of telocentric fragments.

The occurrence of misdivision may explain the arising of B-series with a Ctl-chromosome instead of a whole C-chromosome (cf. HUSKINS, 1946) but will thus also supply a simple explanation of the C het speltoids which have in a few cases been observed in B-series (NILSSON-EHLE, 1921; LINDHARD, 1922, 1923, 1927), if they are supposed to be of the 40 + C + Cts type. More importance may, however, be laid upon the possibility of explaining the origin of the most common type of subcompactoids (40 + C + Cil). Here the present writers suggest *a misdivision of the C-univalent in a PMC followed by a non-disjunction of the two Ctl-chromatids at the pollen mitosis* as the possible mechanism producing the iso-chromosome (Cil).

The first case of iso-chromosomes resulting from misdivision is that of the secondary trisomes observed in *Datura* by BELLING and BLAKESLEE (1924). Among the progeny of a maize having a telocentric fragment RHOADES (1938, 1940) found plants with an iso-chromosome. The explanation of the origin of iso-chromosomes by misdivision is given by DARLINGTON (1939, 1940), based on his observations in *Fritillaria*. DARLINGTON and THOMAS (1941) found an iso-chromosome among the B-chromosomes of *Sorghum*. Iso-fragments produced by misdivision have further been found by MÜNTZING (1944, 1945, 1946) in *Secale*.

The suggestion that the Cil-chromosome in subcompactoids is produced by misdivision and non-disjunction seems quite in harmony with the genetic analysis of the B-series segregates. The most frequent elimination of misdivided C-univalents is, according to Table 4, found

in series III. This series also gives the lowest frequency of subcompactoid segregates (ÅKERMAN and MAC KEY, 1948). Series I is intermediate both with regard to elimination of misdivided chromosomes and to subcompactoids in het speltoid segregation, while series II is quite reverse to series III. The elimination of the misdivided C-chromosomes follows the total number of C-univalents excluded. Misdivision is observed to occur in about the same frequency in the three series. The comparatively rare segregation of subcompactoids out of B het speltoids ($< 0{,}5\ \%$) may be explained by the high degree of elimination of Ctl- or Cil-chromosomes and by a low functioning capacity of the 20 + Cil pollen. This last assumption is quite in harmony with the low fertility of subcompactoid and compactoid plants.

As Ctl-chromosomes seem to be observed very rarely in the progeny of B het speltoids, the telomitic Ctl-fragment doubtless has a rather high capacity of non-disjunction at the pollen mitosis. Such a supposition is quite in agreement with the observations of HASEGAWA (1934) and MÜNTZING (1945) at pollen mitosis of rye with extra fragment chromosomes. The investigations of MÜNTZING may also give an explanation as to why the fourth possible type, an iso-chromosome with a double Cts-arm, has never been observed, as would otherwise have been expected in view of the mechanism suggested for the origin of subcompactoids. He found (1946) that the small iso-fragment, primarily originated from the short arm of the standard fragment, showed no evidence of non-disjunction, while the large iso-fragment, derived from the long arm of the standard fragment, has a very high ability of postmeiotic non-disjunction. The conditions are, however, not quite the same as in the present case.

According to the misdivision observed and the distribution and elimination of the C-chromosome found at the different meiotic phases of a B het speltoid, the following types of pollen grains (or more correctly generative pollen nuclei) may be formed: 1. (20). — 2. (20 + C). — 3. (20 + Cts). — 4. (20 + Ctl). — 5. (20 + Cil). — 6. (20 + 2 C).

The behaviour of the C-univalent during meiosis and the probable origin of the different types of pollen grains are schematically illustrated in the diagram (Fig. 46). The composition of the centromere is presumed to be in accordance with the ideas recently put forward by ÖSTERGREN (1947). On the strength of the doubleness in the chromosomal spindle fibre bundle attached to the centromere, he considers that the actively mobile component of the centromere is the proximal heterochromatin corresponding to the number of chromatid arms present.

TABLE 5. *Formation of the different types segregated from B het speltoids.*

♀ \ ♂	20	20 + C	20 + Cts	20 + Ctl	20 + Cil	20 + 2 C
20	40 B hom sp	40 + C B het sp	40 + Cts ?	40 + Ctl B het sp	40 + Cil Subnormal	40 + 2 C Normal
20 + C	40 + C B het sp	40 + 2 C Normal	40 + C + Cts C het sp	40 + C + Ctl Subnormal	40 + C + Cil Subcomp.	40 + 3 C Subcomp.
20 + Cts	40 + Cts ?	40 + C + Cts C het sp	40 + 2 Cts C hom sp	40 + Cts + Ctl ?	40 + Cts + Cil ?	40 + 2 C + Cts ?
20 + Ctl	40 + Ctl B het sp	40 + C + Ctl Subnormal	40 + Cts + Ctl ?	40 + 2 Ctl Subnormal	40 + Ctl + Cil Subcomp.	40 + 2 C + Ctl ?
20 + Cil	40 + Cil Subnormal	40 + C + Cil Subcomp.	40 + Cts + Cil ?	40 + Ctl + Cil Subcomp.	40 + 2 Cil Comp.	40 + 2 C + Cil Subcomp.
20 + 2 C	40 + 2 C Normal	40 + 3 C Subcomp.	40 + 2 C + Cts ?	40 + 2 C + Ctl ?	40 + 2 C + Cil Subcomp.	40 + 4 C Comp.

If the same types of female gametes are produced, as is very probable, the formation of the different types segregated from B het speltoids would be as shown in Table 5.

As can be observed, several of the potential segregates have not yet been found, but these unknown types have certainly a very low probability of arising and perhaps some of them may be one of the types that LINDHARD found as segregates in his B-series, namely the perennis, the dwarf normal, the dwarf speltoid, or the late maturing type.

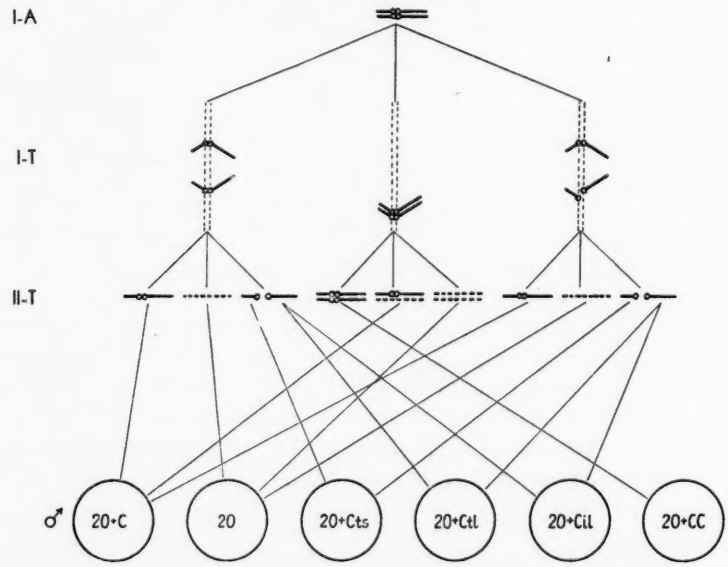


Fig. 46, diagram showing the behaviour of the C-univalent and the probable origin of the different types of pollen grains of B het speltoids. At telophase II only one of the poles is taken into consideration in order to avoid the diagram becoming too complex.

The authors think that it is also possible that the direct appearance of the subcompactoids in normal *Tr. vulgare* may be due to a misdivision of one of the C-univalents that are occasionally found in normal wheat. The observation of subcompactoids in C-series (ÅKERMAN and MAC KEY, 1948) may be given a similar explanation.

For a confirmation of the non-disjunction really occurring, further investigations devoting special attention to the pollen mitosis will be necessary.

SUMMARY.

The behaviour of the C-chromosome during the meiosis of B het speltoids from three different series has been studied with special attention to the distribution of the univalent-halves at telophase II and their elimination in the tetrads.

Misdivision of the submedian C-chromosomes was observed at both first and second telophases, more frequently at the second. Misdivision is supposed to be the mechanism producing B-series with a C-chromosome lacking the shorter arm and to be the explanation of the rare changes from B- to C-type previously observed by other authors. The C-type is here thought to be the most extreme one with a short telocentric chromosome.

A misdivision of the C-univalent in a PMC of a B het speltoid followed by a non-disjunction at the pollen mitosis of the two chromatids, derived from the long arm, will be one possible mechanism for producing subcompactoids out of the B speltoid series. The occurrence of subcompactoids in C-series and directly from normal wheat may be given a similar explanation.

The formation of the different types of gametes and the complicated segregation from B het speltoids are explained as a consequence of the formation of new chromosomes by misdivision.

LITERATURE CITED.

1. BELLING, J. and BLAKESLEE, A. F. 1924. The configurations and sizes of the chromosomes in the trivalents of 25-chromosome *Daturas*. — Proc. Nat. Acad. Sci., Vol. 10, pp. 116—120.
2. DARLINGTON, C. D. 1939. Misdivision and the genetics of the centromere. — Journ. of Genetics, Vol. 37, pp. 341—364.
3. — 1940. The origin of isochromosomes. — Journ. of Genetics, Vol. 39, pp. 351—361.
4. DARLINGTON, C. D. and THOMAS, P. T. 1941. The activity of inert chromosomes in *Sorghum*. — Proc. of the Roy. Soc. of London, Vol. 130, pp. 127—150.
5. HASEGAWA, N. 1934. A cytological study on 8-chromosome rye. — Cytologia, Vol. 6, pp. 68—77.
6. HILLARY, B. B. 1940. Uses of the Feulgen reaction in cytology. II. New techniques and special applications. — Bot. Gaz., Vol. 102, pp. 225—234.
7. HOLLINGSHEAD, L. 1932. The occurrence of unpaired chromosomes in hybrids between varieties of *Triticum vulgare*. — Cytologia, Vol. 3, pp. 119—141.
8. HUSKINS, C. L. 1928. On the cytology of speltoid wheats in relation to their origin and genetic behaviour. — Journ. of Genetics, Vol. 20, pp. 103—122.

9. HUSKINS, C. L. 1946. Fatuoid, speltoid and related mutations of oats and wheat. — Bot. Rev., Vol. 12, pp. 457—514.
10. HUSKINS, C. L. and SPIER, JANE D. 1934. The segregation of heteromorphic homologous chromosomes in pollen mother cells of *Triticum vulgare*. — Cytologia, Vol. 5, pp. 269—277.
11. HÅKANSSON, A. 1930. Zytologische Beobachtungen an s. g. Speltoidheterozygoten beim Weizen. — Sv. Bot. Tidskr., Vol. 24: 1, pp. 44—57.
12. — 1932. Zytologische Studien an compactoiden Typen von *Triticum vulgare*. — Hereditas, Vol. XVII, pp. 155—196.
13. — 1940 a. Die Meiosis bei verschiedenen Mutanten von *Godetia Whitneyi*. — Lunds Univ. Årsskrift, N. F. Avd. 2, Bd. 36, Nr. 5, pp. 3—37.
14. — 1940 b. Die Meiosis bei haploiden Pflanzen von *Godetia Whitneyi*. — Hereditas, Vol. XXVI, pp. 411—429.
15. KOLLER, P. C. 1938. Asynapsis in *Pisum sativum*. — Journ. of Genetics, Vol. 36, pp. 275—305.
16. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas, Vol. XXVIII, pp. 177—211.
17. LINDHARD, E. 1922. Zur Genetik des Weizens. Eine Untersuchung über die Nachkommenschaft eines im Kolbenweizen aufgetretenen Speltoidmutanten. — Hereditas, Vol. III, pp. 1—90.
18. — 1923. Fortgesetzte Untersuchungen über Speltoidmutationen. Begrannungs-Komplikationen bei *Compactum*-Heterozygoten. — Hereditas, Vol. IV, pp. 206—220.
19. — 1927. Über Ährendichte und Spaltungsmodi der Speltoidheterozygoten. — Kgl. Vet. Landb. Aarsskr., 1927, pp. 1—37.
20. LOVE, R. M. 1938. A cytogenetic study of white chaff off-types occurring spontaneously in Dawson's Golden Chaff winter wheat. — Genetics, Vol. 23, p. 157.
21. MÜNTZING, A. 1930. Einige Beobachtungen über die Zytologie der Speltoid-mutanten. — Bot. Not., 1930, pp. 35—47.
22. — 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas, Vol. XXX, pp. 231—248.
23. — 1945. Cytological studies of extra fragment chromosomes in rye. II. Transmission and multiplication of standard fragments and iso-fragments. — Hereditas, Vol. XXXI, pp. 457—477.
24. — 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. — Hereditas, Vol. XXXII, pp. 97—119.
25. NILSSON-EHLE, H. 1917. Untersuchungen über Speltoidmutationen beim Weizen. — Botaniska Notiser, 1917, pp. 305—329.
26. — 1920. Multiple Allelomorphe und Komplexmutationen beim Weizen. — Hereditas, Vol. I, pp. 277—311.
27. — 1921. Über mutmassliche partielle Heterogamie bei den Speltoidmutationen des Weizens. — Hereditas, Vol. II, pp. 25—76.
28. NISHIYAMA, I. 1931. The genetics and cytology of certain cereals. II. Karyogenetic studies of fatuoid oats with special reference to their origin. — Jap. Journ. of Genetics, Vol. 7, pp. 49—101.

29. RHOADES, M. M. 1938. On the origin of a secondary trisome through the doubling of a half-chromosome fragment. — Genetics, Vol. 23, pp. 163—164.
30. — 1940. Studies of a telocentric chromosome in maize with reference to the stability of its centromere. — Genetics, Vol. 25, pp. 483—520.
31. UCHIKAWA, I. 1941. Genetic and cytological studies of speltoid wheat. II. Origin of speltoid wheat. — Mem. Coll. Agric. Kyoto Imp. Univ., 50, pp. 1—64.
32. UPCOTT, M. 1937. The external mechanics of the chromosomes. VI. The behaviour of the centromere at meiosis. — Proc. of the Roy. Soc. of London, Series B, Vol. 124, pp. 336—361.
33. ÅKERMAN, Å. and MAC KEY, J. 1948. A genetical analysis of some speltoid strains. — Hereditas, Vol. XXXIV, pp. 301—320.
34. ÖSTERGREN, G. 1947. Proximal heterochromatin, structure of the centromere and the mechanism of its misdivision. — Botaniska Notiser 1947, pp. 176—177.

CONTENTS.

I. Observations in PMC's of het B speltoids 323
Metaphase I 323
Anaphase I 323
Telophase I 324
Interkinesis 326
Metaphase II 326
Anaphase II 326
Telophase II 326
Tetrads 328
II. Observations in PMC's of subcompactoids 328
III. Discussion 329
Summary 335
Literature cited 335

Communications from the Swedish Seed Association. No. 11.

SPONTANEOUS PHYSIOLOGICAL MUTATIONS IN OPHIOSTOMA

BY *NILS FRIES*
INSTITUTE OF PHYSIOLOGICAL BOTANY OF THE UNIVERSITY OF UPPSALA, SWEDEN

INTRODUCTION.

IN connection with experiments on the effect of various chemicals on the mutation frequency in the fungus *Ophiostoma multiannulatum*, some puzzling observations were made which led to the investigation here described. In the experiments mentioned, conidia were suspended in a nutrient solution lacking one ingredient necessary for growth. From these cultures — called starvation cultures — samples were taken at certain intervals and plated out on agar containing a complete nutrient solution. It turned out that in these platings a fair number of conidia developed into mycelia with an appearance deviating more or less from the normal. The percentage of such divergent mycelia seemed to increase with the age of the conidia in the starvation culture.

Since a possibility seemed to exist that the divergent mycelia represented mutations induced by the lack of an indispensable growth factor, I considered the phenomenon worth investigating.

METHODS.

All the experiments described below were intended to record the changes which might occur in the composition of conidial suspensions of *Ophiostoma multiannulatum*. These suspensions were either growing ones, or so-called starvation cultures, where growth was prevented by the absence of a necessary constituent of the nutrient solution. In all experiments only certain significant *physiological* properties of the conidia and the mycelia developed from them were considered, since it is easier to characterize and compare these properties than the morphological ones, and the isolation technique used here made the grouping of the conidia according to such properties particularly simple. Thus all conidia could be selected which differed from the normal type in not being able to develop a mycelium (or new conidia) in the ordinary synthetic nutrient solution, but which could develop on a medium containing malt- or yeast-extract. Such conidia and 1-conidial

mycelia had lost the capacity of performing some biosynthetic process necessary for the production of living matter from the few, simple constituents of the synthetic medium.

As the basic solution, the following one was exclusively used, called »Modified medium 3» [almost identical with »Medium 3» (FRIES, 1938) but without NH_4NO_3]: glucose 20 g, NH_4-tartrate 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0,5 g, NaCl 0,1 g, $CaCl_2$ 0,1 g, $ZnSO_4 \cdot 7H_2O$ 4,43 mg, $MnSO_4 \cdot 4H_2O$ 4,05 mg, $FeCl_3 \cdot 6H_2O$ 4 mg and distilled water 1 litre. Except when mentioned otherwise, the vitamins B_1 (40 γ per l.) and B_6 (40 γ per l.) were added to this solution, as both of them are necessary growth factors for *Ophiostoma multiannulatum*.

In *normally growing cultures*, modified medium 3 was used with an addition of 5 % malt extract and 10 mgs of inositol per litre. The cultures were grown in Jena glass tubes of dimensions 18 × 155 mm., and each containing 10 cc. nutrient solution. When inoculated with the mycelium in question (from a stock culture on malt agar or some other standard substrate) the tube was put into a shaker. In 2 or 3 days the inoculum had developed into a dense suspension of actively budding conidia, more than 10 million per cc., and with a percentage germination of almost 100. If the culture was used as an *inoculation culture*, it was filtered through cotton wool so as to be freed from single hyphae present, and then washed at least twice by centrifugation.

The *starvation cultures* were also tubes with 10 cc. liquid nutrient solution, placed in a shaker. Each tube was inoculated with c. 10 million conidia from an inoculation culture prepared in the way just described. The solution lacked one ingredient indispensable for the growth of the conidia (except for the case of No. 11 which lacked two ingredients). For *No. 11* (wild type) the basic solution without vitamins was used, and for the other strains, *Nos. 870*, *1174*, *1202* and *1204*, all of which required hypoxanthine or adenine, the same medium with the vitamins was employed. Thus in the first case B_1 and B_6, in the latter cases the necessary purin, were the factors limiting growth in the starvation cultures.

To find the percentage of physiologically divergent conidia in the cultures, samples had to be plated out from them onto a complete agar medium. One, or a half cc., was removed from the shaken culture tube, diluted to a suitable degree with sterile water, and the conidial suspension obtained in this way was finally mixed with melted malt-yeast-extract agar (2,5 % malt-extract + 0,5 % yeast-extract, »Marmite» + 1,5 % agar-agar) and poured into petri dishes. The dilutions

were performed in such a way that at least one series of 30 or 40 plates could be obtained with not more than 40 germinating conidia in each one. In this rather complete agar medium, wild type conidia, as well as several different sorts of deviating conidial types, were enabled to develop into small mycelia, which were isolated and physiologically examined. The technique used will be described in more detail in a further paper.

All experiments were performed at a temperature of + 25° C.

The following strains of *Ophiostoma multiannulatum* were used in the experiments:

No. 11: wild type;

No. 870: hypoxanthine- or adenine-requiring;

No. 870 a, b and *c*: do., each strain derived from one single conidium of No. 870;

No. 1174: adenine-requiring;

No. 1174 a: do., inoculation culture obtained directly from a single conidium of 1174;

No. 1202: adenine-requiring;

No. 1204: adenine-requiring.

All strains belong to the same mating type, +.

As *mutants* are designated those physiologically deviating mycelia which have been derived from conidia of one of the strains listed above. These strains — except No. 11 — are themselves originally mutants (obtained by x-raying), a fact, however, that for the sake of simplicity is left out of consideration in the following.

EXPERIMENTS.

1. *The occurrence of physiological mutants in normally growing cultures and in starvation cultures.* — In Table 1 the results from 10 experiments are presented in which 1-conidial mycelia were isolated from normally growing conidial cultures of seven different strains of *Ophiostoma multiannulatum*. In some cases these cultures have also been used for the inoculation of the starvation cultures to be discussed later.

As might have been expected, a high percentage of germination was established in all cases, often practically 100 %. No exact determinations of the percentage germination were, however, made. In all, 21721 1-conidial mycelia were isolated and examined. Of these, six proved to be physiological mutants, namely 1 hypoxanthine-less from

TABLE 1. *Isolation of mutants from normally growing cultures.*

Experiment No.	Strain No.	Days in shaken tube culture	Number of 1-conidial mycelia isolated	Number of mutants	% mutants	Requirements of the different mutants
1	11	3	1597	1	0,06	1 hypoxanthine
2	11	4	885	0	0	—
3	11	4	1295	0	0	—
4	870	5	2277	1	0,04	1 reduced sulphur
5	870	5	2980	4	0,13	1 arginine 2 lysine 1 unknown factor
6	870 c	2	2240	0	0	—
7	1174	3	3317	0	0	—
8	1174 a	6	2009	0	0	—
9	1202	4	1655	0	0	—
10	1204	4	3466	0	0	—

No. 11, and 1 parathiotrophic, 1 arginine-less, 2 lysine-less, and 1 requiring a hitherto unidentified amino acid from No. 870. Among the 10447 1-conidial mycelia from Nos. 1174, 1202 and 1204 none were physiologically deviating.

In the experiments with starvation cultures, 29316 1-conidial mycelia were altogether isolated. As can be seen in Table 2, a relatively large number of physiologically deviating mycelia, in all 119, were found among them.

From No. 11, only 2 mutants were obtained, both requiring inositol. The starvation culture was here only 3 days old, and the germination percentage was high.

The three tests which were made from a starvation culture of No. 1174 (experiment No. 17) after 7, 16 and 24 days, respectively, led to the isolation of 46 mycelia differing from No. 1174 in their physiological characters. One of them was no doubt a true mutant, requiring pantothenic acid; as to the others, however, I do not dare to label them as mutants without further examination. In all these cases it seemed to be a question of a disturbance of the amino acid synthesis, of a kind not previously observed in *Ophiostoma*. The mycelia did not grow at all, or grew very poorly, in the basic medium (with B_1, B_6 and adenine); on the other hand, good growth occurred when hydrolyzed casein was added to the medium. No specific amino acid, however, was required, and comparatively good growth could be obtained with arginine *or* methionine, but still better with these amino acids together. A genetic analysis is necessary to decide whether it is a question of mutation or

TABLE 2. *Isolations of mutants from starvation cultures.*

Experiment No.	Strain No.	Limiting factor in the starvation culture	Days in starvation culture	Number of 1-conidial mycelia isolated	Number of mutants	% mutants	Requirements of the different mutants
11	11	$B_1 + B_6$	3	4996	2	0,04	2 inositol
12 a	870	Hypoxanthine	10	2618	15	0,57	11 arginine
							2 methionine
							1 asparagine
							1 nicotinic acid
12 b	870	»	16	1645	5	0,30	5 arginine
13 a	870	»	4	563	10	1,78	9 methionine
							1 p-amino-benzoic acid
13 b	870	»	11	3159	23	0,73	2 arginine
							19 methionine
							1 reduced sulphur
							1 nicotinic acid
14 a	870 a	»	4	457	0	0	—
14 b	870 a	»	10	1048	2	0,19	1 arginine
							1 lysine
15 a	870 b	»	4	3852	5	0,13	3 arginine
							1 lysine
							1 uracil
15 b	870 b	»	10	670	3	0,45	1 lysine
							1 reduced sulphur
							1 unknown factor
16 a	870 c	»	10	1402	2	0,14	1 lysine
							1 histidine
16 b	870 c	»	20	2069	1	0,05	1 lysine
17 a	1174	Adenine	7	453	1	0,22	1 pantothenic acid
					(+2)	(0,66)	(2 arginine-methionine)
17 b	1174	»	16	1370	(23)	(1,68)	(23 »)
17 c	1174	»	24	519	(20)	(3,85)	(20 »)
18	1174 a	»	10	1808	1	0,06	1 arginine
19	1202	»	8	2270	1	0,04	1 lysine
20	1204	»	13	417	3	0,72	2 inositol
							1 unknown factor

not. Until that is made, I will not record these 45 mycelia among the mutants, the number of which then amounts to 74.

On the other hand, there is no doubt that the physiologically deviating, 1-conidial mycelia isolated from starvation cultures of Nos. 870, 870 a, 870 b, 870 c, 1174 a, 1202, and 1204 are real mutants. They are all parallel cases to the earlier studied x-ray induced mutants, and they represent the majority of the mutant types which are earlier known in *Ophiostoma* (cf. FRIES, 1947). Twelve different types of

physiological mutations are represented here (see Table 2), among them single cases of asparagine- and histidine-heterotrophy, which are very rare in x-ray treated material.

At the first surprising results of the starvation culture experiments, I was inclined to assume that mutations were induced in these cultures, possibly as a consequence of the absence of hypoxanthine or adenine, which could be supposed to cause disturbances in the nucleic acid synthesis. It soon turned out, however, that such an interpretation of the results was not very likely. In comparing the mutant yields from the two experiments No. 12 and No. 13, one can find some interesting differences. In the two tests (»platings»), a and b, made in experiment No. 12, the arginine-requiring mutants, 11 and 5, respectively, are predominant, while only a few methionine-requiring mutants, 2 and none, respectively, were obtained. In the two tests of experiment No. 13, on the other hand, the arginine-requiring mutants are very few, none and 2, respectively, but the methionine-requiring ones represent the great majority with 9 and 19 mycelia, respectively. This distribution cannot be brought about by mere chance. The two experiments were performed in the same way, except that the tests were taken and plated out after a different number of days in the different tests. This difference cannot, however, reasonably be responsable for the divergent results of the two experiments. It is difficult to imagine that a direction of the mutation processes should have occurred in experiment No. 12 towards arginine-heterotrophy, and in experiment No. 13 towards methionine-heterotrophy. Different inoculation cultures were, however, used for the two experiments. In my opinion the simplest explanation seems to be to assume a different composition of the inoculation cultures: in the first inoculation culture (for No. 12) a great number of arginine-less conidia, and in the second inoculation culture (for No. 13) a great number of methionine-less conidia were present. These conidia of different physiological character could be the result of spontaneous mutations in the two inoculation-cultures or possibly further back in the stock culture of No. 870 from which the inocula for the inoculation cultures were taken.

Assuming this, the mutations isolated from Nos. 870 a—c must have occurred rather lately, and in the case of No. 1174 a, where the inoculation culture was started from a single conidium, the mutant obtained must be the result of a mutation in one of the c. 25 cell generations in the inoculation culture.

As appears from the isolation experiments with normally growing

conidial cultures (identical with inoculation cultures), mutant conidia there are very rare. To explain the relatively abundant occurrence of mutants in the isolations from starvation cultures, it is necessary then to assume that in such cultures a selection takes place in favour of mutant conidia, in such a way that the »normal» conidia die faster than the mutant ones. Unfortunately no exact determinations were made of the percentage germination in the starvation cultures. Only in seven cases were approximate determinations made, giving values between 2 and 12 %.

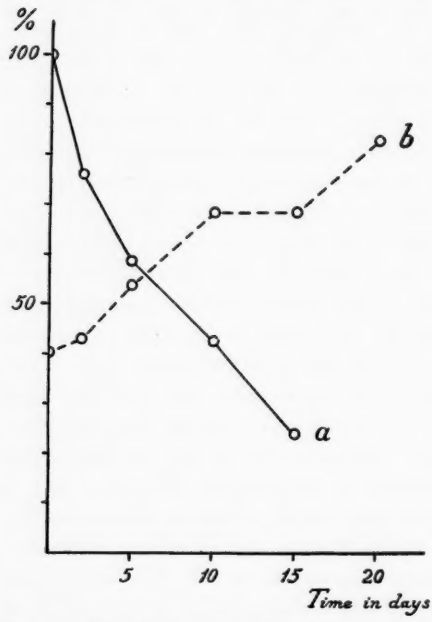


Fig. 1. The first reconstruction experiment (Table 3). — *a*: percentage living conidia of all conidia; *b*: percentage living mutant conidia (No. 1747) of all living conidia.

The validity of this proposed interpretation of the experimental results can obviously be tested by starvation culture experiments in which a known percentage of the conidia at the beginning represents a mutant type. Two such reconstruction experiments were made.

2. *Reconstruction experiments.* — In both of the reconstruction experiments the same combination of conidia was used, namely conidia from No. 870 (hypoxanthine-less) and from No. 1747 (hypoxanthine-arginine-less), the latter of which was originally obtained as a mutant from the former one in experiment No. 12 b. The two components thus represented two different degrees of heterotrophy.

The first experiment was arranged in the following way. Inoculation cultures of No. 870 and No. 1747 were washed several times by centrifuging and then they were mixed so as to form a conidial suspension with c. 10 million conidia per cc. A culture tube with modified medium 3 was inoculated with this suspension. A count proved that the culture contained 950000 conidia per cc. An examination of conidia plated out from this culture showed that 59,8 % of the conidia represented No. 870,

TABLE 3. *The first reconstruction experiment. The viability of two sorts of conidia in starvation culture.*

Days in starvation culture	Number of living conidia analysed	Number of living conidia per cc. (and %) Altogether		No. 870		No. 1747		Living conidia of No. 1747 in % of all living	Living conidia: the ratio No. 1747 / No. 870
0	400	950000	(100,0)	568100	(100,0)	381900	(100,0)	40,2	0,7
2	400	720000	(75,8)	412600	(72,6)	307400	(80,5)	42,7	0,7
5	140	555000	(58,4)	258100	(45,4)	296900	(77,7)	53,5	1,1
10	300	402000	(42,3)	127400	(22,4)	274600	(71,9)	68,3	2,1
15	160	222500	(23,4)	70980	(12,5)	151500	(39,7)	68,1	2,1
20	211	—	—	—	—	—	—	82,9	4,8

and 40,2 % No. 1747. The germination percentage was practically equal to 100.

This starvation culture was then placed in a shaker for 20 days, and at certain intervals samples were taken out from it, diluted and plated out onto a complete agar medium. In this way the percentage germination of No. 870 as well as of No. 1747 was determined. The result of these tests are recorded in Table 3 and Fig. 1.

The total germination power of the conidia in the starvation culture decreased slowly and after 15 days it was 23,4 % against the original 100 %. However, considerably more conidia of No. 870 than of No. 1747 had died, viz. 87,5 % and 60,3%, respectively. The test after 20 days proved a failure, so that only the proportion between the two sorts of living conidia could be calculated. No. 1747 now represented 82,9 % of all living conidia, against originally 40,2 %.

The experiment consequently indicated a much greater mortality (shorter length of life) for the less heterotrophic conidia of No. 870 than for No. 1747.

The second experiment was arranged in such a way that the state of affairs in the starvation cultures dealt with in the foregoing section was reproduced even more accurately. Inoculation cultures were started of No. 870 and No. 1747, in both cases with a single conidium as the initial inoculum. The same numbers of conidia from each culture were mixed together and the mixture was poured out on the slanted malt agar surface in a culture tube. Two days later a rich conidia-producing mycelium had developed in the tube, and a new inoculation culture was started with an inoculum taken from the surface of this malt agar culture. As was expected, both types of conidia developed in this new

TABLE 4. *The second reconstruction experiment. The viability of two sorts of conidia in starvation culture.*

Days in starvation culture	Number of living conidia analysed	Number of living conidia per cc. (and %) Altogether	No. 870	No. 1747	Living conidia of No. 1747 in % of all living	Living conidia: the ratio No. 1747 / No. 870
0	800	1200000 (100,0)	394800 (100,0)	805200 (100,0)	67,1	2,0
5	800	420000 (35,0)	103300 (26,2)	316700 (39,3)	75,4	3,1
15	800	165000 (13,8)	25570 (6,5)	139430 (17,3)	84,5	5,5
25	840	127400 (10,6)	4841 (1,2)	122559 (15,2)	96,2	25,3
35	840	96000 (8,0)	1728 (0,4)	94272 (11,7)	98,2	54,5

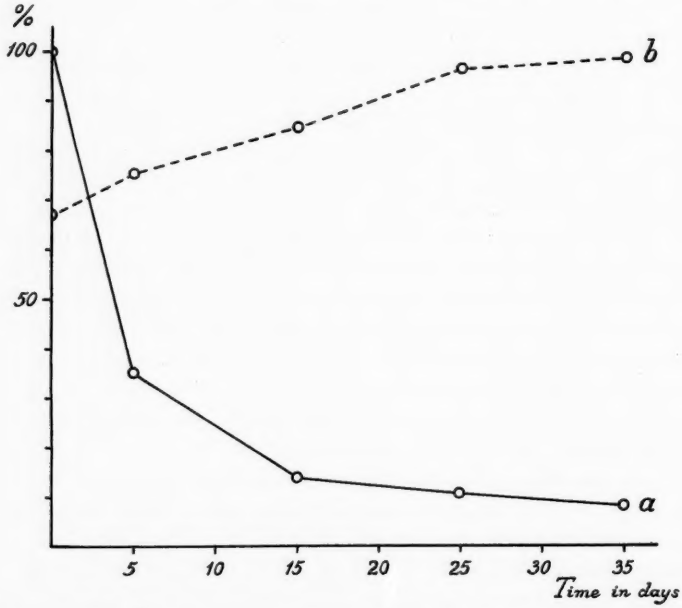


Fig. 2. The second reconstruction experiment (Table 4). — *a*: percentage living conidia of all conidia; *b*: percentage living mutant conidia (No. 1747) of all living conidia.

inoculation culture, 32,9 % represented No. 870, and 67,1 % No. 1747. This inoculation culture might be looked upon as a reconstruction of the inoculation cultures mentioned in the preceding paragraph, where the percentage of mutants, however, was originally unknown (although, of course, much lower than 67,1 %!). A tube with modified medium 3 was inoculated with a duly filtered and washed suspension of conidia

from this culture, and the germination power of the conidia was examined repeatedly (see Table 4 and Fig. 2).

Even more obviously than in the first experiment, it appeared that the conidia of No. 870 were more short-lived than those of No. 1747. When the experiment was finished after 35 days, only 1728 living conidia of No. 870 per cc. remained against 94272 of No. 1747, which corresponds to a decrease of 99,6 % in the former case, and 88,3 % in the latter, from the original 100 %. The proportion between the two kinds of conidia, No. 870 : No. 1747, which at the beginning was 1 : 2, after 35 days had changed to 1 : 55.

If the proportion between the two types of conidia in this experiment had originally — in the inoculation culture — been 10000 : 1, i. e. 0,01 % of No. 1747 (instead of 67,1 %), it would have been necessary to isolate a great number of 1-conidial mycelia to have the chance of picking up a mutant. In a starvation culture, however, started from such an inoculation culture, the proportion between the two types of conidia would have gradually changed rather considerably, if the mortality of the conidia is assumed to be the same as in the last mentioned experiment. Instead of having one mutant per thousand conidia, in 35 days one would have 27 per thousand, which means that the probability of isolating a mutant conidium from the starvation culture has considerably increased.

The results of the two reconstruction experiments, particularly the last one, correspond quite well with the assumption that the increasing percentage of mutants among the living conidia in a starvation culture is due to a selective process.

DISCUSSION.

From the last two experiments described, it is evident that the mutant No. 1747 is more long-lived than its mother strain No. 870 in conidial starvation cultures. Although this case is the only one thoroughly studied, I am, however, inclined to assume that the result might be extended to be valid for all physiological mutants spontaneously arisen in No. 870 as well as in the other strains of *Ophiostoma multiannulatum*. In that way a generally applicable interpretation should be conceivable of the peculiar differences as to the percentage of mutants in normally growing, and in starvation cultures.

Thus, the great majority of the mutants, at least, should not have been induced in the starvation cultures, but they represent the results

of spontaneous mutations which have occurred at different occasions, unknown in point of time, before the inoculation of the starvation cultures in question. If this interpretation is accepted, it is of course impossible to indicate the real number of mutations which is the basis of the yield of mutant conidia. All the 18 arginine-requiring mutants in the experiments Nos. 12 and 13 may be the descendants of one and the same mutant conidium which arose several cell generations ago. The arginine-requiring mutant derived from No. 1174 a (in experiment No. 18), on the other hand, must have arisen independently of the just mentioned ones, either in the 1-conidial inoculation culture

TABLE 5. *Spontaneous physiological mutations obtained from the different strains of Ophiostoma multiannulatum.*

Accessory growth factor required	No. of the mother strain	Number of mutants obtained	Minimum number of mutations
Arginine	870, 870 a, 870 b, 1174 a	24	4
Methionine	870	30	1
Lysine	870, 870 a, 870 b, 870 c, 1202	8	5
Asparagine	870	1	1
Histidine	870 c	1	1
Reduced sulphur	870, 870 b	3	2
Hypoxanthine	11	1	1
Uracil	870 b	1	1
Inositol	11, 1204	4	2
Pantothenic acid	1174	1	1
Nicotinic acid	870	2	1
p-Amino-benzoic acid	870	1	1
Unknown factors	870, 870 b, 1204	3	3
(Arginine-methionine?)	1174	(45)	(1)
In total		80 (+ 45)	24 (+ 1)

or possibly in the starvation culture. Thus, starting from the history of the different cultures, one can indicate certain minimum figures for the number of mutations which occurred. From Table 5 it is evident that even if one accepts these minimum figures as representative of the real frequency of spontaneous mutations, those mutant types are most frequent which were also the most numerous ones in the earlier experiments with x-ray irradiation (FRIES, 1947).

It is not in itself a very remarkable fact that special external conditions can bring about a selection of a certain type of mutation. The cases studied here are, however, peculiar in so far as the selection seems to work quite unspecifically: all sorts of mutations are favoured

against the mother strains. It is perhaps too bold at such an early stage of the analysis of this problem to try to produce an explanation. In my opinion, however, the following interpretation seems quite conceivable, and might at least serve as a working hypothesis for continued investigations.

The only thing characterizing all of the mutants obtained from the starvation cultures is that they represent a higher degree of heterotrophy than the mother strains. In a substrate where the absence of an indispensable growth factor (in the widest sense of the word) makes growth impossible the viability of the cells might be directly proportional to the degree of heterotrophy. In a nutrient solution, lacking for instance vitamin B_6 (necessary for all strains), the conidia of No. 11 (wild type) would consequently be more short-lived than those of No. 870 (hypoxanthine-less), and these last-mentioned ones would be more short-lived than those of No. 1747 (hypoxanthine-arginine-less), etc.

It is a well-known fact that the resistance of a cell against various unfavourable physical and chemical influences is on the whole greatest when resting, i. e. when the processes of metabolism and of energy turnover have stopped, or are going on very slowly. This is, for instance, the case at low temperatures or under dry conditions. A heterotrophic organism like a fungus (or a single fungus cell) requiring a number of organic substances in its substrate, is forced to rest, when these substances are absent. It seems to be a reasonable assumption that the rest becomes deeper and at the same time the resistance greater, if the degree of heterotrophy increases. This would then imply that the physiological mutants, all of which are more heterotrophic than the mother strains, are also more viable than those.

The working hypothesis here suggested will now be tested on various mutant strains of *Ophiostoma multiannulatum*. Should the hypothesis be corroborated by these experiments several interesting consequences can be developed; if not, it will be necessary to search for another explanation of the peculiar phenomena which were the basis of this investigation.

My hearty thanks are due to my wife, Mrs. LISBETH FRIES, Fil. Lic., and to Miss ANNA PANDERS, who have rendered me the most valuable assistance in this work. The investigation has been carried out with the aid of a grant from Statens naturvetenskapliga forskningsråd, for which I wish to express my sincere gratitude.

SUMMARY.

(1) Five different strains of *Ophiostoma multiannulatum*, No. 11 (wild type), No. 870 (hypoxanthine-less), No. 1174, No. 1202, and No. 1204 (all 3 adenine-less), were grown in conidial culture in shaken tubes. From these cultures single conidia were isolated, and the developing mycelia were physiologically examined. Out of a total of 51037 such mycelia, 125 proved to be physiologically deviating from the mother strains, and at least 80 of them could be looked upon as true mutants. These represented 13 different types, requiring arginine, methionine, lysine, asparagine, histidine, reduced sulphur, hypoxanthine, uracil, inositol, pantothenic acid, nicotinic acid, p-amino-benzoic acid, or some still unknown factor. The same types are already known in *Ophiostoma*, having been obtained through x-ray irradiation of conidia, and, apparently, with about the same relative frequency.

(2) Of the isolated 1-conidial mycelia, 21721 had been obtained from normally growing conidial cultures, and 29316 from so-called starvation cultures, where the absence of one indispensable constituent made growth impossible. In the former case 6 (0,03 %), in the latter 74 (0,25 %) — or possibly 119 (0,41 %) — mutants were obtained.

(3) The cause of the abundant occurrence of mutant conidia in the starvation cultures was investigated. The distribution of the mutant types amongst the different experiments showed that the majority, at least, could scarcely be assumed to have been induced in the starvation cultures, but must have been present in them from the beginning, i. e. in the growing cultures from which the inoculum was taken.

(4) Two so-called reconstruction experiments showed that conidia of the hypoxanthine-arginine-less type arisen from a hypoxanthine-less strain were considerably more long-lived in starvation culture than the conidia of the mother strain. The results of these experiments thus supported the assumption of a selection of spontaneous mutations, not an induction of mutations, going on in the starvation cultures.

(5) Finally, as a working hypothesis, it was proposed that the viability of conidia is proportional to their degree of heterotrophy.

LITERATURE CITED.

1. FRIES, N. 1938. Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. — Symb. Botan. Upsal., III, No. 2: 1—188.
2. — 1947. Experiments with different methods of isolating physiological mutations of filamentous fungi. — Nature, 159: 199.

SPONTANEOUS CROSSING BETWEEN DIPLOID AND TETRAPLOID SINAPIS ALBA

BY *GÖSTA OLSSON* AND *BRITA RUFELT*
SWEDISH SEED ASSOCIATION, SVALÖF

RECENTLY, MÜNTZING (1948) has shown that tetraploid rye, when grown in yield tests together with a diploid standard variety, is markedly less fertile and yields less than when the same tetraploid is grown by itself, isolated by distance from any diploid. Evidently, in tests where both types grow on adjacent plots the tetraploid cannot develop the whole of its capability of seed production, since a large number of its egg-cells are fertilized by pollen from the diploid, resulting in triploid and abortive embryos.

It is of interest to find out whether tetraploid strains of white mustard (*Sinapis alba*) are influenced in a similar manner by the close vicinity of diploids in a yield test including both types. In order to study this question experiments were made in 1946 and 1947, so arranged as to give information about the effect of diploids upon the yield of tetraploids. The experiments also offered opportunities for a comparison between diploid, triploid and tetraploid mustard as regards the size of the seed.

I. MATERIAL AND METHODS.

In the experiments the common diploid white mustard ($2n = 24$) grown in Sweden has been tested together with a tetraploid ($2n = 48$) obtained in 1941 by the colchicine method. The experiments of 1946 included one plot of ten rows of each of the diploid and the tetraploid and also a plot of twenty rows in which diploid and tetraploid alternated. In 1947 the plots of diploid and tetraploid had twenty rows each, and the plot with alternate rows included 50 rows. Furthermore, the experiment, a scheme of which is given below, was replicated so that 20-row diploid and tetraploid neighbour plots were replicated four times, fifty-row alternate plots twice. The rows were 40 cm apart and each row had a length of 5 metres, the area of each row thus being 2 square metres. In 1946 20—25 plants in each row were harvested separately and studied as regards crossing and seed-development. In

Row 20 — 1 1 — — — 20 1 — — — — — — — — — — — 50 20 — — — 1 1 — — 20

Diploid	Tetraploid	Alternate rows Rows 1, 3, 5, etc., diploid Rows 2, 4, 6, etc., tetraploid	Tetraploid	Diploid

Diploid	Tetraploid	Alternate rows Rows 1, 3, 5, etc., diploid Rows 2, 4, 6, etc., tetraploid	Tetraploid	Diploid

Fig. 1. Scheme of the 1947 experiments.

1947 20 plants were taken at random from each of the rows 1, 2, 5, 10, 15, 20 within each 20-row plot of the tetraploid, in row 2 of the corresponding diploid plots and in some of the rows of the alternate-row-plots. Furthermore, every row was harvested separately and the yield of seed determined. In order to study the degree of crossing when pollen from diploids is abundant, one row of tetraploids was sown in a large field (1,5 hectares) of diploids, and from this row 25 plants were taken at random for a determination of the degree of crossing. The effect of distance upon the percentage of crosses was studied in an experiment in which plots of tetraploid white mustard were planted at varying distances from a large field (4 hectares) of common diploid white mustard.

The chromosome numbers were determined in root-tips of seedlings germinated on moist filter paper. The number of chromosomes was determined with an accuracy sufficient to distinguish with certainty between diploid, triploid and tetraploid numbers.

II. THE INFLUENCE OF THE DIPLOID UPON THE YIELD OF THE TETRAPLOID.

The variation in the yield of the tetraploid with increasing distance from a diploid is evident from the graph on Fig. 2. The dotted line represents the results from 1946, the continuous line those from 1947. The results from 1946 represent only five rows at each end; they are more irregular than those from the experiment in 1947, the latter representing a mean of four replicates.

It is evident that the yield of the tetraploid is much lower in rows

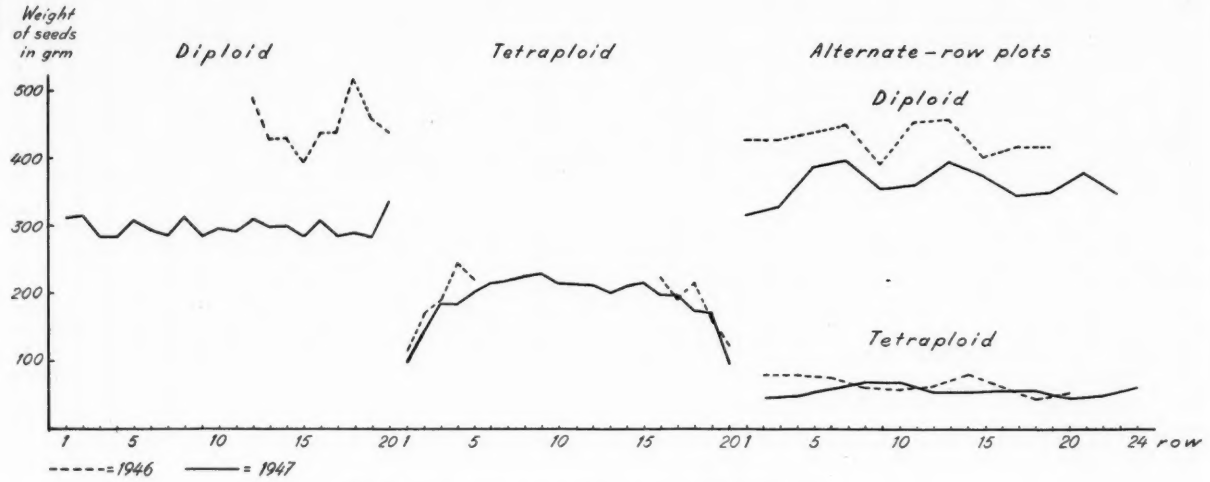


Fig. 2. The yield of diploid and tetraploid white mustard.

adjacent to the diploid than in other rows, whether the diploid occurs as a 10—20-row plot or as a single row in the plot of alternate rows. In rows where the neighbour is diploid the yield in 1946 is 115—122 gms, in 1947 95—96 gms, the corresponding yield of rows from the middle of the tetraploid plots is 224 and 213 gms, respectively.

When a tetraploid row is surrounded on both sides by diploid rows the yield is still lower in the tetraploid. In 1946 the average yield of ten such rows was $65{,}2 \pm 4{,}33$ gms and in 1947 the average of 24 rows was $55{,}2 \pm 2{,}42$ gms, whereas the yield per row of the diploid from the same experiments was $428 \pm 7{,}16$ gms and $358{,}6 \pm 6{,}50$ gms, respectively. Neither in the plots with alternate rows nor in the larger plots of diploid (see Fig. 1) is there any decrease in yield in the immediate vicinity of tetraploids (see Fig. 2). Instead, in 1947 the highest yield was obtained from the diploid row next to the tetraploid plot and the diploid in the alternate-row-plot yielded more than in the plot of pure diploids. There is no corresponding difference in 1946, however, and most likely the results in 1947 depend upon differences in vegetative development. This year the experiment was sown rather late and the drought was severe, and the tetraploid suffered relatively more from the adverse conditions than the diploid.

III. THE SIZE OF THE SEED.

HOWARD (1939) found that triploid seeds obtained from the cross tetraploid $\times$ diploid kale (*Brassica oleracea*) were much smaller than the seeds of either parent. In tetraploid mustard grown close by diploid there occurred very small seeds, which proved to be triploid. In determining the degree of intercrossing this fact was utilized. Each plant selected for the study was thrashed separately and the seeds were divided into different fractions by letting them pass through a series of sieves with round holes of the following diameters in mm: 3,5, 2,6, 2,0, 1,7 and 1,4. Each size-fraction was carefully studied as to the shape of the seed, and after the chromosome number had been determined on a large number of seeds it was possible for experienced persons to subdivide the size-fractions into subfractions according to the type of the seed.

Nearly all seeds from the tetraploid passed through the sieve with the largest holes. The large well-developed seeds remained on the 2,6 mm sieve, whereas the smaller tetraploid seeds passed through this sieve also. The seeds on the 2,6 sieve were fairly uniform in type, the

TABLE 1. *The result of dividing seeds into fractions by means of sieves.*

Diameter of the holes in the sieves in mm	Seeds from tetraploid plants						Seeds from diploid plants					
	Frequency in per cent	Weight of 1000 seeds, gms	Germination percentage	Number of chromosome-determined plants	2n	Classification used in text	Frequency in per cent	Weight of 1000 seeds, gms	Germination percentage	Number of chromosome-determined plants	2n	Classification used in text
2,6	71,91	11,77	98	102	48	Round, large; tetraploids	13,01	9,78	97	49	24	Round, large; diploids
	13,36	7,60	93	87	48 [1]	Round, small; tetraploids						
	8,05	5,30	58	190	93 % 48 7 % 36	Angular-flattened	79,60	6,19	98	34	24	Round, normal; diploids
	0,63	5,28	95	79	54 % 48 46 % 36	Round, small; mixed						
2,0	3,23	3,28	95	72	36	Round, large; triploids	6,95	3,13	95	80	24	Round, or flattened; small diploids
1,7	2,14	2,21	98	39	36	Round, medium size; triploids	0,44	1,87	30	29	24	Often angular or flattened; very small diploids
1,4	0,68	1,42	69	51	36	Somewahtangular; small triploids						

[1] One plant 36.

average weight of 1000 seeds was 11,77 gms and 102 seeds, in which the chromosome number was determined, were all tetraploid. (Table 1 and Fig. 3.)

The fraction obtained on the 2,0 mm sieve was by far the most heterogeneous one. It included the small well-developed tetraploid seeds already mentioned, some still smaller, round and smooth seeds

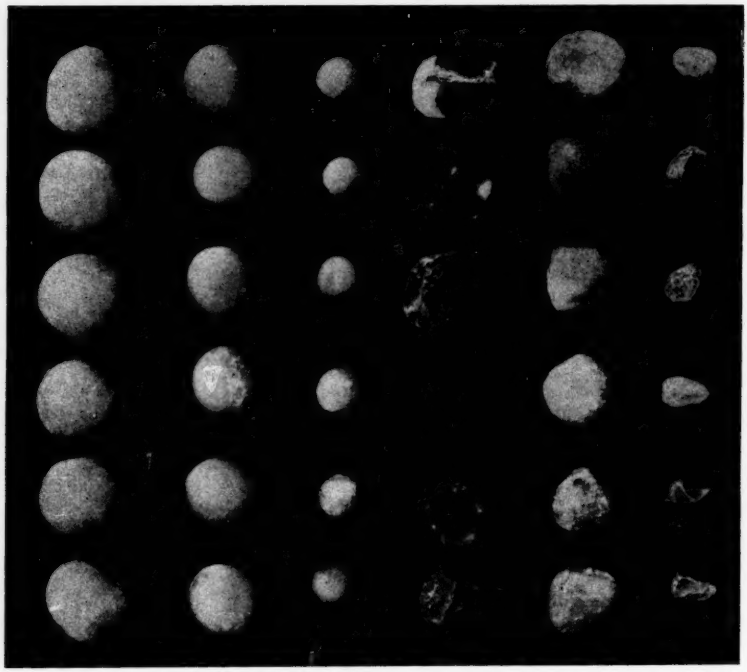


Fig. 3. From left to right: tetraploid, diploid, triploid, shrivelled, angular-flattened and abortive seeds.

and, finally, a number of irregularly shrivelled seeds. It was possible, however, to subdivide the fraction by the type of the seeds. One subfraction contained seeds very similar to the tetraploids in the 2,6 mm fraction though somewhat smaller, the average weight of 1000 seeds being 7,60 gms. The chromosome number was determined on 87 seeds of this subfraction; with one exception they were tetraploid, and in determining the degree of intercrossing all seeds in this subfraction have been counted as tetraploid. Another subfraction was made up of smaller

but round and smooth seeds, the average weight of 1000 seeds being 5,28 gms. Chromosome counts of 79 seeds in this subfraction showed that 46 per cent had the triploid number, 54 per cent the tetraploid, and in determining the percentage of intercrossing 46 per cent of the seeds in this subfraction have been counted as triploids. The 2,0 mm fraction, finally, included some angular, flattened and poorly developed seeds. Together with these, into a subfraction denoted as »angular-flattened», some seeds were assigned which could not with certainty be grouped into any of the previously mentioned subsections. The seeds of the subfraction angular-flattened had an average weight of 5,30 gms per 1000 seeds; the germination was poor (on an average 58 per cent) and 93 per cent of the germinated seeds (Table 1) had the $\pm$ tetraploid number of chromosomes. Several of the seeds in this subfraction seem to be aneuploids with a chromosome number of $4x \pm 1$—4, but further investigations are necessary to settle this point, since an exact count was difficult in many cases because of chromosome stickiness. MÜNTZING (1943), when studying tetraploid rye, found that shrivelled and poorly developed seeds were as a rule aneuploids.

The fraction obtained on the 1,7 mm sieve included a small number of angular seeds, similar to those in the »angular-flattened» subfraction just discussed. Seeds of this type have been counted together with similar ones from the 2,0 mm fraction. Most of the seeds in the 1,7 mm fraction were round and smooth, however, with an average weight of 3,23 gms per 1000; chromosome numbers were determined on 72 such seeds and in all cases the number was triploid.

All seeds in the 1,4 mm fraction were very similar to those just discussed but smaller, with an average weight per 1000 seeds of 2,21 gms; all of 39 determined seeds were triploid. A number of seeds passed through even the 1,4 mm sieve. They were well-filled but somewhat angular, the average weight of 1000 seeds was 1,42 gms, the germination was good and all of 51 seeds studied were triploids.

Of the tetraploid seeds, 77,2 per cent have a diameter between 2,6 and 3,5 mm, 78,4 per cent of the triploids fall between 1,4 and 2,0 mm. The average diameter of the tetraploids is 2,88 mm, that of the triploid 1,76 mm. The mean weight per 1000 seeds of the well-developed tetraploids is 11,12 gms, if the angular-flattened seeds are included the value is 10,62 gms. The mean for the three clearly triploid fractions is 2,69 gms per 1000 seeds. In calculating these mean values the number of seeds in each fraction is taken into account as well as the average weight of seeds in each.

Besides the more or less normally developed seeds so far discussed there are two morphologically distinct types of abnormal seeds. One of these types has been called shrivelled seeds (see Fig. 3), and the seeds belonging to this type are best described as empty spheres, more or less shrivelled or shrunk. There are transitions between this type and the one classified as »angular-flattened». Most of these seeds are found on the 2,0 mm sieve but they occur fairly frequently in other fractions as well. The weight of 1000 seeds is 2,78 gms and the seeds do not germinate. The second type consists of very small and flattened seeds, all of which pass through the smallest meshed sieve. The weight per 1000 is as low as 0,20 gm and none of these »abortive seeds» germinate.

When seeds from diploid plants were passed through the series of sieves, a few of them remained on the 2,6 mm sieve but the majority were obtained in the 2,0 mm fraction (see Table 1). A small number of seeds came in the 1,7 mm fraction and a few passed through even this sieve. Most of the seeds passing through the 2,0 mm sieve were angular or flattened and were evidently small, poorly developed diploid seeds, but some of them were round and smooth and morphologically very similar to triploid seeds in the same fractions. The chromosome number was determined on 109 seeds of this type from diploid parents; all of them had the diploid number. The large seeds from the diploid might be tetraploids, resulting from unreduced gametes. Counts of the chromosome number on 49 seeds showed them to be true diploids, however. Thus, all well-developed seeds from the diploid plants were diploid with an average 1000-seed weight of 6,43 gms. The diameter of 79,6 per cent of the seeds was between 2,0 and 2,6 mm, the average being 2,37 mm. The diploid also yielded some seeds classified as shrivelled, with a weight of 1,79 gms per 1000 and a small number of abortive or unfertilized seeds with a weight of 0,17 gm per 1000.

It is evident that the method used for dividing the seeds into several fractions by means of sieves is sufficiently accurate for a separation of tetraploid and triploid seeds. The method is less adequate for a separation between tetraploid and diploid or between triploid and diploid, even if a classification by the morphological type of the seed gives better results than a classification by size only. There is a possibility of some diploid seeds being overlooked in the progeny of tetraploids, the same applying to tetraploid or triploid seeds in the diploid. The number of such seeds, however, cannot be great, since none were found among those seeds for which the chromosome number was determined, in spite of a special search being made for such exceptions.

The results show that if diploid and tetraploid mustard are grown close by one another under field conditions there occur a number of crosses between the two types, with the tetraploid as a mother. The tetraploid plants will, therefore, yield not only tetraploid seeds but also well-developed triploid seeds, smaller than both tetraploid and diploid ones, and also a large number of abortive seeds. In artificial crosses between tetraploid and diploid, with the tetraploid as a mother, the result is again small triploid seeds and a large number of abortive ones. The reciprocal cross does not yield any triploid seeds but a number of empty shells of the triploid size. These results are in good agreement with those obtained by HOWARD.

IV. THE DEGREE OF INTERCROSSING BETWEEN TETRAPLOID AND DIPLOID.

TEDIN and NISSEN (1932) have studied the pollen distribution in a field of turnips. There is a considerable difference between plants in regard to the percentage of vicinism, and the authors consider these differences to be not only random but due to actual biological differences. There are genetical differences in the tendency to selfing and there are such differences as plant height, time of flowering, etc., genetical and environmental. Similar factors have caused a great variation between plants in the present material in the degree of vicinism. It is, however, possible to obtain a fair estimate of degree of vicinism in the tetraploid at different distances from the diploid, using the number of seeds in the fractions and subfractions discussed to determine the number of tetraploid and triploid seeds in each case. The percentages of intercrossing given in Tables 2 and 3 are the means for 4000 to 8000 seeds from 18—20 plants, taken at random, and the means are weighted with regard to the number of seeds or embryos per plant. As was to be expected, the frequency of triploid seeds decreases with an increase in the distance from the diploid. In Table 2 the number of triploid seeds is given in per cent of the total number of developed seeds as well as in per cent of the total number of embryos. There is a considerable difference between the replicates in 1947 but, nevertheless, the differences between rows 1, 2 and 5 are highly significant ($P < 0{,}001$). The fact that samples taken from the harvest of whole rows give results which coincide with the mean values of the individually thrashed plants tends to strengthen the value of the results (Table 2).

In the tetraploid row next to the diploid the percentage of triploid

TABLE 2. *Percentage of triploid seeds in the experiment on vicinism in 1947.*

Row No.	Distance from diploid in metres	Triploids in percentage of developed seeds: Mean value of 20 plants					Mean value from samples of whole rows; four replicates	Triploids in percentage of total number of ovules; mean of four replicates
		I	II	III	IV	Mean		
1	0,40	12,7	19,7	19,5	15,0	16,7	15,3	6,4
2	0,80	15,4	6,8	8,7	11,8	10,7	8,5[1]	5,3
5	2,00	5,6	1,8	4,1	1,5	3,3	—	2,0
10	4,00	4,9	2,3	1,9	2,6	2,9	1,7	2,0
15	2,00	2,2	5,7	2,4	1,5	3,0	—	2,0
20	0,40	14,4	26,0	14,0	—	18,1[1]	13,2[1]	9,0[1]

[1] Mean of three replicates.

TABLE 3. *Per cent abortive seeds in the experiment of 1947.*

Row No.	Distance from diploid in metres	Abortive seeds in percent of total numbers of ovules: I	II	III	IV	Mean
1	0,40	53,0	47,1	47,5	48,0	48,9
2	0,80	35,4	33,2	37,2	40,7	36,6
5	2,00	27,3	25,6	22,5	27,2	25,7
10	4,00	19,9	22,1	20,9	25,5	22,1
15	2,00	19,6	22,2	21,5	22,9	21,6
20	0,40	37,8	36,1	40,7	—	38,2[1]

[1] Mean of three replicates.

seeds was 16,7 in 1947, the corresponding percentage was 10,7 in row 2, 3,3 in row 5 and 2,9 in row 10, towards the diploid row in the alternate-row-plot the percentage is then again increasing. The figures obtained in 1946 show the same tendency, even if the actual percentage values are somewhat different. As was to be expected, the percentage of triploid seeds in the tetraploid rows in the alternate-row-plots is still higher. The mean for 10 such rows in 1946 was 43,3 per cent of the developed seeds and in 1947 the mean was 34,9 per cent. The row of tetraploids, sown in a large field of diploids, gave no less than 73,4 per cent triploids among the developed seeds.

The relative frequency of seeds classified as angular-flattened has

also been determined in different rows, but there is no evident influence from the diploid. The percentage of germination in this group, however, increases with increased distance from the diploid and it is possible that some of the non-germinating seeds in this group have resulted from pollination by pollen from the diploid. The frequency of angular-flattened seeds was higher in 1947 than in 1946, a result of the high frequency of late branches in 1947 on which the seed did not ripen before harvest. The frequency of shrivelled seeds is not influenced by the distance from diploid plants, but was again higher in 1947 than in 1946. Some of the »seeds» in this class might be embryos, aborted in a relatively late stage of development. The high frequency in 1947, however, makes it probable that most of them are prematurely harvested seeds from late branches.

The frequency of aborted seeds, again, is dependent upon the distance from the diploid. In per cent of the total number of ovules it was 48,9 per cent in the first row as against only 22,1 per cent in the tenth row in 1947 (Table 3). In the alternate-row-plot the values for three rows are 64,2, 60,9 and 64,9, or 63,3 on an average. The data from 1946 are, unfortunately, somewhat incomplete, but as far as they go they agree with those from 1947. In the single tetraploid row in the diploid field 72,0 per cent of the seeds were abortive.

The plots of tetraploid mustard which were planted at varying distances from a diploid field were, unfortunately, rather weak in development. Further, scattered plants of diploid mustard appeared on the northern and eastern sides of the field at various distances. The results from this experiment are somewhat unreliable, but they do not contradict the results of the other experiments. The frequency of developed triploid seeds was remarkably low, but the percentage of abortive embryos shows a marked decrease with increasing distance from the diploid field (Table 4).

TABLE 4. *Per cent abortive seeds in tetraploid mustard, grown at different distances from a large field of diploid mustard.*

Distance from diploid field	Per cent of abortive seeds				
	Southern border	Western border	Northern border	Eastern border	Mean
0,50 metres	59,0	37,1	35,3	40,9	43,1
6,25 »	19,1	10,2	17,0	25,1	17,9
12,50 »	10,4	9,1	9,8	21,6	12,7

The results indicate that the cross between tetraploid and diploid mustard results not only in germinable triploid seeds but also in a large number of cases in abortive embryos, whose development is interrupted at a very early stage. This seems to be the only reasonable explanation of the fact that the percentage of such abortive embryos decreases with increasing distance from the diploid. It is, however, by no means certain that all the »abortive seeds» are a result of a tetraploid × diploid cross. Embryos with an essentially tetraploid number, lacking several chromosomes or having a corresponding number of supernumerary ones, may be abortive. It is also possible that this category includes a certain number of cases where no fertilization has taken place. The frequency of abortive seeds in the tetraploid when no influence from the diploid was involved was studied by thrashing twenty plants from an isolated field of tetraploid mustard and dividing the seed into fractions by the methods used in dividing the seeds from the experiments. In this material there were 4 per cent of abortive seed but also 0,3 per cent of triploid seeds. There is evidently some haploid pollen even in a tetraploid field, which may have derived from isolated diploid admixtures and from distant diploid fields. The value 4 per cent for abortive seeds is rather low in comparison with the values obtained in the vicinism experiments, 22,1 per cent in the tenth row and 12,7 per cent in plots which were 12,5 metres distant from a diploid field. The general development in the experimental plots, however, was distinctly weaker than in the field of tetraploids from which the value 4 per cent was obtained, and most probably a tetraploid field with the same weak growth would have shown a larger percentage of abortive seeds. That considerably more than 4 per cent of abortive seeds are the result of fertilization within the tetraploid is also indicated by the values obtained from different rows in the vicinism experiment.

The frequency of developed triploid seeds in percentage of total number of ovules drops from 6,4 to 2,0 per cent between rows 1 and 10 and from 9,0 to 2,0 per cent between rows 20 and 10, indicating a drop in the frequency of haploid pollen in a ratio of about 3 : 1 and 4,5 : 1, respectively. TEDIN and NISSEN (1932) obtained the value 6 : 1 for the relative frequencies of foreign pollen in row 1 and a row 4,2 metres from the border line. When the frequency of abortive seeds drops from 48,9 per cent in row 1 to 22,1 per cent in row 10 and from 38,2 per cent to 22,1 per cent between rows 20 and 10, this indicates that about 15 per cent of such seeds are the results of intra-tetraploid pollination.

As previously mentioned, no developed triploid seeds were found on the diploid plants in the experiments on vicinism. Among the seeds classified as »shrivelled» there were, however, some small empty shells similar to those obtained when artificially crossing diploid with tetraploid, and it seems probable that they are the result of a similar cross. This type, however, was very rare and, since it was not possible to distinguish it accurately from other types of shrivelled, the frequency was not determined. The total frequency of the group »shrivelled» was independent of the distance from tetraploids. The frequency of »aborted seeds» in the diploid has been studied on a comparatively small material. On the whole the frequency is low, reaching 4,5 per cent in the second row from the tetraploid in 1947, and it seems to be independent of the distance from the tetraploid.

V. DISCUSSION.

An attempt has been made to calculate the effect upon yield of the occurrence of small triploid seeds and of abortive seeds. The variation in yield between different tetraploid rows is at least mainly caused by the variation in the occurrence of triploids — developed or abortive.

The percentage of triploids increases with an increase in the relative frequency of haploid pollen; in 1947 it was 16,7 per cent in the border row of the tetraploid plot and 34,9 per cent in the plot of alternate rows and in 1946 it was 73,4 per cent when a single row of tetraploids grew in a field of diploids.

In the border row of the tetraploid plot there were, in per cent of total number of ovules, 6,4 per cent triploid seeds and 48,9 per cent abortives, about 30 per cent of which may safely be counted as a result of fertilization with haploid pollen, giving a percentage of 35—40 for intercrossing between tetraploid and diploid. In their study TEDIN and NISSEN (1932) found only 18—19 per cent of intercrossing in the border row and that only about 40 per cent of the pollen derive from a distance of more than 0,3 metres. In their case the two varieties had different flower colours (different shades of yellow) and, since the plant in question is pollinated by insects, this fact may tend to reduce the percentage of vicinism. On the other hand, each plant in a row has other plants in the same row as closest neighbours and it is improbable that 40 per cent of the available pollen should derive from the neighbouring rows at one side. There is no definite proof that haploid pollen has an advantage over diploid at the fertilization. But, if the percentage of crosses

reaches about 40 per cent, it seems probable that the haploid pollen has some advantage over the diploid, which again may depend upon a more rapid growth of the haploid pollen tubes. Investigations concerning the growth of pollen tubes of different kinds are in progress.

The fact that well-developed triploid seeds occur only when the tetraploid is used as a mother is explained by WATKINS (1932) and HOWARD (1939, 1942, 1947) on the assumption that normal seed development is possible only when there is an appropriate relation between the chromosome numbers of mother plant, endosperm and embryo. It seems probable that the chromosome relation embryo/endosperm is of importance for the development of triploid seeds in mustard and that this relation allows normal seed to be developed only when the tetraploid is used as a mother. The possibility cannot, however, be overlooked that the diploid pollen from the tetraploid is unable to penetrate the tissues of the diploid style. The rather high frequency of empty seeds of triploid size in the cross diploid $\times$ tetraploid indicates, however, that diploid pollen functions to a considerable extent.

A proper embryo/endosperm relation in chromosome number is not, however, sufficient to warrant a good development of triploid seeds. In most cases the majority of the triploid embryos become abortive but in individual plants the percentage of developed triploid seeds may be as high as 40—50 per cent, indicating that in these cases nearly all triploid embryos have developed normally. The chromosome relation between embryo and endosperm is the same in all cases and the great differences between plants indicate that the ability of the triploid embryos to develop is also very much dependent upon the genic constitution of the gametes involved.

SUMMARY.

The degree of intercrossing between diploid and tetraploid white mustard (*Sinapis alba*) under field conditions has been studied. The results allow the following conclusions:

(1) The yield of the tetraploid is abnormally low if diploid plants grow in the neighbourhood.

(2) The deleterious effect of the diploid upon the yield of the tetraploid depends upon the formation of triploid embryos, the majority of which are abortive, while some develop into triploid seeds.

(3) The triploid seeds are much smaller than either diploid or tetraploid seeds.

The investigation was carried out at the Oil Plant Section and the Cyto-Genetic Laboratory of the Swedish Seed Association and in close collaboration with the Institute of Genetics. We wish heartily to thank the Chiefs of these institutes, Dr. G. ANDERSSON, Dr. A. LEVAN and Professor A. MÜNTZING for valuable help and advice during the work. We are also much indebted to Dr. O. TEDIN for many good ideas and for translating the manuscript. Finally, we desire to tender our thanks to the Margarine Factories Sales Company for valuable financial support granted to us.

LITERATURE CITED.

1. HOWARD, H. W. 1939. The size of seed in diploid and autotetraploid *Brassica oleracea* L. — Journ. of Genetics 38: 325—340.
2. — 1942. The effect of polyploidy and hybridity on seed size in crosses between *Brassica chinensis*, *B. carinata*, amphidiploid *B. chinensis-carinata* and autotetraploid *B. chinensis*. — Journ. of Genetics 43: 105—119.
3. — 1947. Seed size in crosses between diploid and autotetraploid *Nasturtium officinale* and allotetraploid *N. uniseriatum*. — Journ. of Genetics 48: 111—118.
4. MÜNTZING, A. 1943. Aneuploidy and seed shrivelling in tetraploid rye. — Hereditas XXIX: 65—75.
5. — 1948. Några data från förädlingsarbetet med tetraploid råg och rågvete. — Nordisk Jordbrugsforskning 1948.
6. TEDIN, O. och NISSEN, Ö. 1932. Studier över pollendistributionen i ett rovfrö-fält. — Nordisk Jordbrugsforskning 1932: 225—241.
7. WATKINS, A. E. 1932. Hybrid sterility and incompatibility. — Journ. of Genetics 25: 125—162.

CONTENTS.

I. Material and methods 351
II. The influence of the diploid upon the yield of the tetraploid 352
III. The size of the seed 354
IV. The degree of intercrossing between tetraploid and diploid 359
V. Discussion 363
Summary 364
Literature cited 365

Communications from the Swedish Seed Association. No. 13.

ABSTRACTS — KURZE MITTEILUNGEN

ARNE HAGBERG: Hybrid vigour in *Galeopsis*.

This is a preliminary note from an investigation of hybrid vigour in intraspecific crosses in *Galeopsis Tetrahit* and *bifida* and in crosses between these species.

In 1945 MÜNTZING published a paper on hybrid vigour in *Galeopsis Tetrahit*. As index of vigour he used plant height of the F_1 generation relative to the mean of the parents. The parents are pure lines self-fertilized under control since 1924. MÜNTZING found a more pronounced vigour in the partially pollen sterile F_1 generations and a probable correlation between degree of hybrid vigour and pollen sterility. The partial pollen sterility in the F_1 generation is an indication of structural chromosome differences between the parent lines. Thus there seems to be a positive correlation between the degree of structural chromosome differentiation and the degree of genetic diversity. MÜNTZING's data, however, were not quite conclusive and therefore further investigation of this problem was considered desirable.

Since 1945 the present writer is studying hybrid vigour in *Galeopsis* and some of the data obtained bear upon the problem mentioned. MÜNTZING has kindly put material at my disposal.

The parental lines are treated in the same way as the F_1's, i. e. the seed used is produced by artificial crosses. As index of vigour the total dry weight of full-grown plants is used. Pollen fertility is determined in the usual way with aceto-carmine preparations.

The diagram, Fig. 1, shows the correlation between degree of sterility of the F_1 generations and their dry weight relative to the heaviest of the parent lines. Some of the crosses were investigated all the three years (1945, 1946 and 1947), some of them two or only one year. The points in the diagram are marked with different signs indicating different years. The figures at the points refer to the table given of the 17 combinations examined. The square points represent 1947, and this year all the 17 combinations were investigated. The coefficient of the correlation was calculated in the material of 1947 and found to be $-0{,}82$. The test of its significance gives a $t = 5{,}48$ and $P < 0{,}001$. This good correlation is not only caused by the very high vigour of the F_1 generations of the crosses between *bifida* and *Tetrahit* lines. If these species-crosses are excluded, the coefficient of correlation will be $-0{,}63$ ($t = 2{,}67$ and $P \sim 0{,}02$), i. e. still a rather good significance.

Most of the F_1 combinations, 13 out of 17, in this investigation were not represented in MÜNTZING's paper of 1945. My results are in good agreement with those of MÜNTZING, and will support his conclusion.

MÜNTZING stated that in the partially pollen sterile F_1 generations seed setting is also slightly reduced and therefore more energy might be left for vegetative growth. This is rather improbable, he thought however, because plant growth seems to be finished with the development of the terminal inflorescence already before the seeds are ripe. According to my observations, F_1 plants with semi-sterile pollen have a rather good seed setting — only about 10 per cent lower than in the fertile plants.

Curves illustrating the development of the plants have been drawn, based on measurements of the plant height at certain intervals. In these curves the differences between the F_1's and the parental lines are often most pronounced during the period *previous* to maturation. When comparing the vigour of the

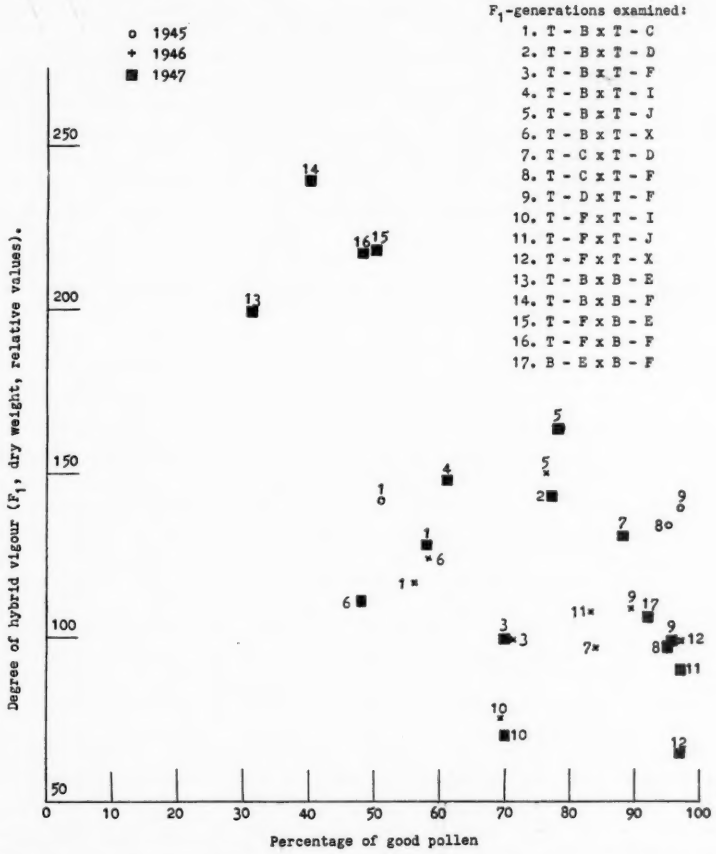

o 1945
+ 1946
■ 1947
F1-generations examined:
1. T - B x T - C
2. T - B x T - D
3. T - B x T - F
4. T - B x T - I
5. T - B x T - J
6. T - B x T - X
7. T - C x T - D
8. T - C x T - F
9. T - D x T - F
10. T - F x T - I
11. T - F x T - J
12. T - F x T - X
13. T - B x B - E
14. T - B x B - F
15. T - F x B - E
16. T - F x B - F
17. B - E x B - F
Degree of hybrid vigour (F1, dry weight, relative values).
250
200
150
100
50
0
10
20
30
40
50
60
70
80
90
100
Percentage of good pollen

Fig. 1. Diagram showing the correlation between degree of sterility and degree of hybrid vigour in F_1 generations in *Galeopsis Tetrahit* (T) and *bifida* (B).

different lines a fixed stage is necessary. It is difficult or impossible to get such a fixed stage during plant development; therefore the point of maturation was chosen.

In MÜNTZING's investigation, as well as in mine, a greater vigour of the semi-sterile F_1 plants as compared with that of the fertile F_1 plants is observed.

This condition cannot be explained by a delayed maturation or by a continued vegetative growth due to sterility.

Dry weight — total production of the plant — is a better index of vigour than height. But even in height this correlation between sterility and hybrid vigour is evident. The hybrid vigour of the F_1 can be measured either in relation to the weight of the best parent as in the diagram, Fig. 1, or to the parental mean. Also in this last case a good correlation is found. Which method of calculation preferred, is a matter of definition of hybrid vigour.

Perhaps, such a general and schematic consideration of this problem cannot give all the truth of it, but an individual treatment and discussion of the single F_1 generations will certainly give more interesting and conclusive results. Later I hope to be able to give more detailed data concerning this and other problems in this investigation.

Institute of Genetics, Svalöf, 10. 2. 1948.

Literature cited.

1. MÜNTZING, A. 1945. Hybrid vigour in crosses between pure lines of *Galeopsis Tetrahit*. — Hereditas, Vol. XXXI, pp. 391—398.

J. A. BÖÖK: A finger-print method for genetical studies.

In genetical studies it is often desirable to register finger-prints. The hereditary characters evaluated from the pattern of the finger-tips may prove to be very useful in question of, for instance, paternity, twin diagnosis and genetic linkage. With the aid of the different blood groups, secretor substance, taste-blindness, etc. it should now be possible to mark several human chromosomes. We are here concerned with traits connected with genes of practically absolute penetrance. Linkage between such genes and hereditary diseases of late manifestation will in certain families make it possible to give a better prognostic statement as to the risk a certain person runs of later falling ill.

In human genetical studies, where often several hundred persons are examined, we need simple and quick methods of registration. We also want to bother our patients as little as possible. This is necessary to ensure joint co-operation.

The ordinary method of making finger-prints is not very convenient in this respect. Its proper application requires a great deal of experience. The family does not like to be painted with ink and the children are more fond of putting their painted fingers on other places than on the examiner's record. If our patients are not able to co-operate, as for instance in the case of severe mental disturbances, the registration is impossible or at least extremely difficult.

The present method, which has been used by the writer, eliminates this trouble and in addition is simple enough to be applied by even an unskilled assistant.

The only things needed are an ordinary transparent cellulose tape and a piece of chalk, white or pale yellow. The tips of the fingers are cleaned with some ether alcohol and painted just a little with the chalk. A bit of the tape

is placed under light pressure on the pattern with the paste-side against the skin. The strip is then removed gently and the finger-print is now recorded. The strips are collected in a special box. As the tapes take a considerable time to dry, it is important not to touch the paste-side.

In the laboratory the finger-prints may be evaluated by placing and stretching the strips under a magnifying glass. They are read against a black background, illuminated from one side.

The strips may also be used as negatives and placed in an ordinary photo magnifier, still with care not to touch the paste-side. They may also be placed in an ordinary 35 mm projector, giving excellent pictures on the screen.

This method is contemplated only as an aid in genetical field work. As such I have found it practical. It allows of a good evaluation of the characters relevant in genetical studies. Whether it would also be useful for other purposes, e. g. in police work, is at present beyond the scope of my experience. Experiments with a view to getting a permanent fixation of the records are in progress.

Dept. of Medical Genetics, Institute of Genetics, Lund University, Sweden.

ANTERO VAARAMA: A triple species hybrid in the genus *Ribes*.

Ribes Culverwellii (= *R. nigrum* × *grossularia*) growing in the Experimental Garden of the State Horticultural Institute (Piikkiö, Finland) has always been completely sterile. The cytological basis of the sterility has been established by MEURMAN (1928). The bush usually forms a number of berries, which, however, are always seedless. It was therefore surprising when in 1940 Mr. O. POHJANHEIMO discovered in a *R. Culverwellii* berry a seed which germinated. In the summer of 1947 the seedling had grown into a bush of 27 cm height and 35 cm breadth, and flowered for the first time. It could then be established with certainty that the seed had developed as a result of open pollination between *R. Culverwellii* and a male plant of *R. alpinum* var. *pumilum* LINDL. growing beside the former plant. The resulting plant was thus a triple species hybrid, which had not previously been found in this genus. Moreover, the parental species belong to different subgenera of the genus *Ribes*, viz. *Berisia*, *Ribesia* and *Grossularia*. Unfortunately the bush, owing to its low viability, was killed by the severe drought in the summer of 1947. The description of this interesting plant specimen is therefore only a short »necropsy report», restricted to certain morphological details.

In regard to its broad habitus as well as to the form of its leaves it most resembles *R. alpinum* var. *pumilum*. The leaves are, however, considerably smaller than in any of the parental species, being 1,0—1,5 cm in breadth and 1,0—2,0 cm in height. The plant had comparatively few leaves. As in *R. Culverwellii*, spines are also lacking in the hybrid.

Flowering is very scarce. The racemes and flowers are upright, each raceme containing 1—3 flowers. In this respect, too, the characters of *R. alpinum* seem to dominate. The flower is of very small size. The sepals are oblong-ovate, nearly upright and concave. Their length is 4 mm, breadth 1,2—

1,5 mm, and they are wine-coloured. The petals are very short, 1,2—1,3 mm long and 0,8—1,0 mm broad. Their colour is yellowish white, the veins greenish. The upper edge of the petal is undulated and narrowly revolute. The stamens are of varying sizes. Their maximum length is 0,6—0,7 mm. The anthers are nearly globose and the filaments ribbon-like. In regard to the size of the petals, sepals and stamens the characters of *R. alpinum* seem to dominate.

The flowers are fully staminate. The pistils are completely undeveloped. This agrees with the classical crossing experiments between dioecious and bisexual plants carried out by CORRENS (1928), which have shown the former character to be dominant.

The pollen is extremely variable in size, as is shown by the following classification:

Size classes (in microns)	5 — 10	— 15	— 20	— 25	— 30	— 35	— 40	— 45	— 50	— 55
Number of grains	2	13	17	28	16	14	6	2	1	1

The great variability of the pollen size indicates a disturbed course of meiosis. The largest grains are equal in size to the grains in the induced tetraploid *R. nigrum* (cf. VAARAMA, 1947, p. 68), suggesting the occurrence of grains with the unreduced chromosome number in the hybrid similarly to *R. Culverwellii* (MEURMAN, 1928). An attempt to cross back the triple hybrid with *R. nigrum* was unsuccessful.

State Horticultural Institute, Piikkiö, Finland.

Literature cited.

1. CORRENS, C. 1928. Bestimmung, Vererbung und Verteilung des Geschlechtes bei den höheren Pflanzen. — Handb. d. Vererb.-wiss. B II c. Berlin.
2. MEURMAN, O. 1928. Cytological studies in the genus *Ribes* L. — Hereditas XI: 289—356.
3. VAARAMA, A. 1947. Morphological and cytological studies on colchicine-induced tetraploid *Ribes nigrum*. — Acta Agr. Fenn.; 67; No. 2: 35—93.